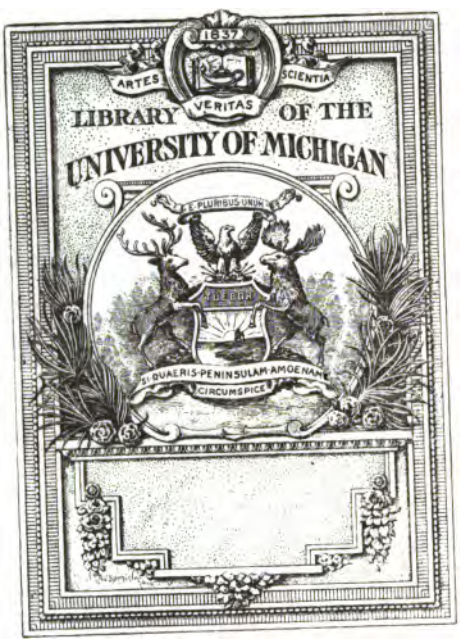
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BIOCHEMIC DRUG ASSAY METHODS

PITTENGER

BIOCHEMIC DRUG-ASSAY METHODS

WITH SPECIAL REFERENCE TO THE PHARMACODYNAMIC STANDARDIZATION OF DRUGS

BY

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PREFACE

This Manual of Biochemic Drug Assay Methods is intended for students of pharmacy, pharmaceutic chemistry and medicine, also for the use of experts engaged in laboratories devoted to drug standardization work.

The data has been collected from monographs, Government bulletins, papers read before medical and pharmaceutical societies, and also from laboratory notes containing the results of the author's original research and observations.

Much of the original data was previously contributed in the form of papers to several national and state medical and pharmaceutical societies, including the American Therapeutic Society, The American Medical Association, The American Pharmaceutical Association and The Pennsylvania State Pharmaceutical Association.

The authors of much of the information on the biochemic assay of drugs appearing in the literature assume that the readers are familiar with the apparatus and technique of the subject. In other words, the literature is written for experts rather than to teach beginners. There is, therefore, a field for a work explaining in detail the methods and apparatus employed for pharmacodynamic standardization. Such a volume is demanded by the rapid advance in the scientific knowledge of drugs as therapeutic agents. It is commencing to be realized by physicians that drugs should be instruments of precision. Chemical assay and standardization is sufficient to render them so when they contain active principles of such character as to permit their identification and isolation in the pure form by chemical methods. But there is another class of drugs not amenable to chemic standardization. Such drugs as digitalis, ergot, cannabis indica, etc., do not lend themselves to standardization by chemical methods. It is with this class of drugs that this volume exclusively deals.

The wants of the pharmaceutical colleges and their students have been considered. Methods familiar to experts, but not referred to in the literature with sufficient detail for students and beginners, are fully described. Apparatus used in the pharmacodynamic labora-

tories of the universities both in Europe and America, is placed before the reader in the form of picture illustrations with explanations as to the use of the same. The illustrations and detailed explanation will enable the student intelligently to follow lectures and demonstrations, and will also prove useful to persons unfamiliar with the subject and unprovided with extensive reference libraries.

Judging from the want of appreciation by the medical profession of the wide variation in the therapeutic activity of drugs (see Table 1, page 6), this Manual, although intended primarily for the use of the pharmacist and pharmaceutic chemist, could be advantageously employed in teaching medical students.

In conclusion, the author takes occasion to acknowledge his indebtedness to Professor F. E. Stewart for his aid in editing the manuscript, to Professor Charles E. Vanderkleed for collaboration in original research, to Dr. Thomas Stotesbury Githens of the Rockefeller Institute for many details in technique, to the Harvard Apparatus Co. of Boston, and C. F. Palmer Co. of London, England, for cuts of special apparatus of their manufacture, and to the H. K. Mulford Company, for laboratory facilities, animals, etc.

P. S. P.

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CHAPTER I

PRELIMINARY CONSIDERATIONS

(1) INTRODUCTORY

The importance of assay methods as a means of securing uniformity in the action of drug preparations has never been fully appreciated. No one has questioned the necessity for preventing gross adulteration and sophistication, but the full meaning of standardization by which drugs are rendered instruments of precision is only beginning to be comprehended.

It must be apparent to any thinking person that adulteration of drugs or their sophistication must of necessity materially influence their physiologic action and therapeutic value. Even the impurities resulting from the processes of preparing medicinal chemicals may seriously modify their therapeutic effects. Variation in active principles in medicinal plants is another factor of importance in considering the question of standardization.

Standardization of drugs may be accomplished either by the use of chemic or pharmacodynamic methods included under the general term pharmaceutic assaying. That the *purpose* of the assay is to secure *uniformity* is admirably set forth by Vanderkleed[1] in the following words:

"*Pharmaceutic assaying* may be defined as the art of determining the amounts of medicinally active constituents of drugs and their preparations. As such it is an exceedingly important link in the chain of progress which is so rapidly binding together medicine and pharmacy under the inclusive science of pharmacology.

[1] From an address delivered by Prof. Chas. E. Vanderkleed before the Alumni of the New York College of Pharmacy, Jan. 11, 1911.

Progress in therapy is largely dependent upon the *uniformity* of the medicinal agents which physicians employ. The absolute necessity for the standardization of pharmaceutic products is therefore apparent. The argument that therapeutists must experimentally determine the proper dosage of an agent to fit the needs of each particular case is no excuse for the tolerance of variation in the strength and potency of the remedy itself; every possible variable should be eliminated in an effort to reduce therapeutics as nearly to an exact science as is possible.

The first requisite for the *chemical* standardization of complex vegetable drugs and their preparations, therefore, is to know exactly what the "medicinally active constituent" or "constituents" are. For this reason it is highly important that pharmaceutic assaying be developed and improved by *the co-operation of the chemist and the pharmacologist.*

It must be remembered that the principal end to be accomplished by the assay of a preparation is to secure a *means of measuring its therapeutic efficiency.* Hence an assay fails of its purpose unless some direct and constant ratio exists between the figures obtained by the assay process and the therapeutic activity.

Having secured such a ratio, the assay process then, and only then, becomes of value. However, when a fairly constant ratio has been established between the results of an assay process and pharmacodynamic activity, such process becomes of unquestioned value—whether or not the constituent actually determined represents the entire activity of the drug, or even whether or not it be the principal medicinal constituent. The physician is not concerned directly with the actual percentage of alkaloid, or glucosid, or resin which a drug or preparation may contain: he is concerned only that the adjustment of a preparation to such a percentage of alkaloid, or glucosid, or resin guarantees to him that this preparation will, under similar conditions, produce the same therapeutic effect that any other sample similarly adjusted will produce. The fact should be emphasized, therefore, that *any assay process* or *any means of standardization* that *tends to establish a uniform ratio between results obtained and therapeutic activity* becomes at once of unquestionable value, and continues to be of value until supplanted by a new or improved process which more closely maintains this ratio."

The purpose of the pharmacodynamic assay, just as of the chemical assay, is to secure a means of measuring therapeutic activity and to make it possible to furnish *uniform* preparations. A satisfactory method which meets these requirements may or may not involve the production of physiologic reactions similar to those for which the drug is intended to be the means of producing when used therapeutically. That the effect chosen as a means of standardization does not parallel the clinical effect sought is not sufficient to condemn the method. It is only necessary that the effect chosen as an earmark is always indicative of a good quality of the drug or preparation, and criticisms of methods on the ground that they are *toxic* methods or that the animal chosen is biologically much different from man are made only

through a lack of conception of the real purpose of the physiologic test, namely, to secure *uniformity*. The determination of the real value of a drug in the treatment of disease in man is another matter entirely.

(2) DEFINITIONS

Pharmacology.—The science that treats of drugs and medicines; their nature, preparation, administration and effect; including Pharmacognosy, Pharmacy, Pharmacodynamics and Therapy-dynamics.

Pharmacognosy treats of the identification and selection of vegetable and animal drugs.

Pharmacy is the science and art of preparing, preserving, compounding and dispensing medicines.

Pharmacodynamics treats of the actions of medicines on healthy organs.

Therapy-dynamics treats of the actions of medicine on diseased organs.

Assaying consists of the quantitative determination of one or more constituents of a product.

Standardization in a general sense means any and all methods for determining and thereby improving the character, quality and strength of materia medica products.

In the specific sense it means the adoption of definite methods and standards and adjusting materia medica products thereto.

While standardization necessarily involves assaying, it also *includes final adjustment to definite strength.*

Pharmacodynamic or physiologic standardization consists in quantitatively determining the potency or drug power upon healthy living tissues and adjusting the product assayed according to a fixed and definite standard of strength.

(3) DEVELOPMENT

Pharmacodynamics is one of the most recent developments of medical science, it being a product of the last half century and more particularly of the last quarter. *Standardization*, however, by pharmacodynamic methods is a product of the last ten to fifteen years and more particularly of the last five.

Since physiologic standardization was first proposed, its importance has become more and more apparent, until to-day all who have carefully looked into the subject agree that its value cannot be overestimated.

(4) HISTORY

The history of standardization may well be divided into five important steps. The *first step* was made by Dr. Lyman Spalding of New York City, who in 1817 submitted to the Medical Society of the County of New York the project for the formation of a National Pharmacopeia. His plan provided that the United States be divided into four districts, in each of which a convention was to be called composed of delegates from all the medical societies, schools, etc., in that district. Each district convention was to form a pharmacopeia and appoint delegates to a general convention in Washington. To this general convention the four district Pharmacopeias were to be taken and from the material thus brought together a National Pharmacopeia was to be compiled.

The adoption of Dr. Spalding's project resulted in the publication of the first National Pharmacopeia on Dec. 15, 1820.

The *second step* toward standardization was the formation and organization of the American Pharmaceutical Association in 1853, the object of which was to improve and regulate the drug market. The necessity for such a step was due to the importation of inferior, adulterated and deteriorated drugs and to the adulteration and sophistication constantly being practised in the United States at the time.

The *third important step* consisted in the adoption of the Purity Rubric and of assay processes for galenical preparations by the Pharmacopeial Convention of 1890.

The *fourth important step* consisted in the securing of legislation known as the Pure Food and Drugs Act of June 30, 1906, by which the standards of the Pharmacopeia were made Law for Interstate Commerce in drugs and medicines.

The *fifth important step* was made by the Pharmacopeial Convention of 1910 in recommending that the Revision Committee adopt *pharmacodynamic* methods for standardizing certain preparations of drugs not amenable to chemical standardization.

(5) DRUGS REQUIRING BIOCHEMIC ASSAY

The list of drugs to which it is necessary to apply biochemic assay methods is not long, since all chemicals are standardized by means of chemical assay. The chemical method of assay is also used for such drugs as opium, belladonna, nux vomica, etc., in which the active constituents are capable of isolation in the *pure form*. There are, however, a number of drugs and their preparations which *cannot* be satisfactorily assayed by chemical methods, either for the reason that their active principles are *not known* or that they *cannot be quantitatively isolated in the pure state* by any of the known chemical methods. In this list are found such important drugs in common use as *digitalis, strophanthus, squills, convallaria, apocynum, aconite, suprarenal extract, pituitary extracts, ergot, cannabis indica, gelsemium,* and *veratrum.* Since there are no satisfactory chemical methods of assay for this list of drugs, recourse must be had to standardization by *pharmacodynamic* means.

(6) VARIATION IN STRENGTH OF NON-STANDARDIZED PREPARATIONS

Variability in the strength of crude drugs has long been a matter of common knowledge; a greater variability in the pharmacodynamic power and therapeutic usefulness of their preparations follows as an inevitable corollary.

Edmunds and Hale quote Fränkel as having reported a variation of from 100 to 275 per cent. in the strengths of infusions of digitalis and 100 to 400 per cent. in the strengths of tinctures of digitalis obtained by him in and around Heidelberg. The following table shows the variation in physiologic activity *before standardization* of some U.S.P. preparations assayed during the past year in the Mulford laboratories.

TABLE I

Drugs	Number assayed	Variation, per cent.
Digitalis, Tr.	51	30 to 444
Digitalis, F.E.	16	26 to 160
Digitalis, S.E.	7	29 to 100
Ergot, F.E.	17	0 to 310
Aconite leaves, Tr.	6	38 to 111
Aconite root, Tr.	12	33 to 363
Aconite root, F.E.	7	52 to 266
Aconite root, S.E.	10	6 to 166
Aconite root, P.E.	11	5 to 100
Cannabis Indica, F.E.	15	40 to 150
Cannabis Indica, S.E.	4	71 to 125
Gelsemium, Tr.	7	64 to 156
Gelsemium, F.E.	7	65 to 220
Gelsemium, P.E.	3	88 to 187
Strophanthus, Tr.	12	55 to 277
Squills, F.E.	13	71 to 153

The above table shows that these *unstandardized* preparations ranged in strength from 0 to 444 per cent. It can be readily understood that with such a variation in the commercial preparations, the physician *cannot*, unless he employs "standardized" preparations, depend upon obtaining a definite effect as the result of a given dosage. If, for example, a druggist who *does not* dispense standardized preparations, fills a physician's prescription, calling for tincture digitalis, with a preparation corresponding in strength to the preparation in the above table which possessed only 30 per cent. of the standard activity, the doctor having failed to obtain the desired effect doubles the dosage. In the meantime, the druggist having replenished his "stock bottle," fills the new prescription with a preparation corresponding in strength to the preparation in the above table possessing 444 per cent. of the standard activity. As a result of this, the patient instead of receiving a dose possessing, theoretically, twice the activity, receives one possessing 28 times the activity of the dose first prescribed.

(7) NECESSITY FOR BIOLOGIC ASSAY METHODS

The above table also illustrates the great necessity for standardization and emphasizes the fact that it is not only essential to know that a drug contains valuable medicinal properties, but that *in order to secure the best therapeutic results these must be present in the commercial preparations in definite and constant amounts.* This can be obtained only by means of *standardization.*

(8) STANDARDIZATION OF CRUDE DRUGS

Standardization of crude drugs is not necessary since, as a rule, crude drugs are not administered as such and also because the use of standardized crude drugs in the manufacture of galenical preparations does *not* insure uniformity in the finished product. It is only necessary to *assay* the crude drugs in order to avoid the use of those which are inert or adulterated. The manufacturer of standardized preparations, however, finds it necessary to *assay* his drugs before purchasing, since the value of the drug to him is in direct proportion to the amount of activity it possesses. If the manufacturer neglected this precaution, he would soon find himself out of pocket.

"Standardized" fluid extracts or tinctures do not necessarily result from the percolation of "standardized" drugs for the following reasons:

First, there is the personal equation of the pharmacist.

Second, though extracted under identical conditions, the resulting preparations may differ in strength.

Third, the variation in the amount of the active principles extracted from the same drug by ethereal or chloroformic solvents and the amount extracted by alcoholic or hydroalcoholic solvents—the therapeutic value of the crude drug is determined by extracting the active principles with ethereal or chloroformic solvents and therefore is not necessarily the same as that of the preparation made from it by percolation with an alcoholic or hydroalcoholic menstruum.

Fourth, the decomposition of some alkaloids produced by the heat employed in concentrating the percolate to a fluid or solid extract.

Aconite is a notable example of number five. The aconitine when subjected to the heat of concentration is often partly split up into its decomposition products—aconine and benz-aconine—which are prac-

tically physiologically inert. Due to this fact it often occurs that a solid or powdered extract made from a very potent drug is almost inactive. For this same reason the chemical assay for powdered and solid extracts of aconite is practically valueless. This assay is based upon the total alkaloidal content, which is determined by titrating the alkaloidal residue with a standard acid solution, making no distinction between the relative amounts of aconitine, aconine and benz-aconine present. It can readily be seen that by this method the inert aconine and benz-aconine will neutralize the standard acid solution and thus give the same percentage results as would be obtained if the residue consisted of active aconitine alone.

It is therefore impossible to prepare standardized fluid extracts, tinctures, etc., from assayed drugs without assaying and adjusting the *finished* products.

(9) DETERIORATION

One of the problems of standardization is to prevent finished products from deterioration. Physicians ask, "of what value is standardization, if the carefully assayed and adjusted preparations, due to deterioration, lose part or all of their activity before being placed in our hands for use?" Fortunately, only a few drugs and their preparations are subject to rapid deterioration. Most of the drugs, such as nux vomica, belladonna, cinchona, opium, etc., have definite alkaloids as their active principles. Preparations of these drugs are quite stable and will, if properly kept, maintain their activity for years.

On the other hand, digitalis, and ergot preparations, *particularly the latter*, deteriorate quite rapidly when exposed to the ordinary conditions under which galenical preparations are kept. In the case of ergot, the activity being greatly impaired, in many instances, in from two to three months.

The subject of deterioration of these drugs has for a long time occupied the minds and attention of various workers but until recently no one had succeeded in determining a method by which manufacturers of preparations of these drugs could put them on the market in a form in which they would remain stable for a definite time.

The principal causes of such deterioration are oxygen of the air, and heat. It is a known fact that fluid extract of ergot retains its activity for a much longer period of time when kept in well-filled and tightly stoppered containers.

To determine the value of a complete exclusion of air, a series of experiments were undertaken by Pittenger and Vanderkleed[1] with fluid extract of ergot.

First of all, this extract was tested by intravenous injections of 0.08 c.c. per kilo into dogs, and gave an immediate rise of blood-pressure represented by 44.8 mm. of mercury. The assay for total alkaloids by the process of Keller gave a percentage of 0.163. This fluid extract of ergot was then divided into four portions, as follows:

A—The first portion was put up in vacuum, in tubes specially designed and made for this purpose.

B—The second portion was filled into bottles which were tightly corked, and allowed to remain for one year unopened.

C—The third portion was filled into bottles which were kept loosely corked for one year, this being obtained by boring a small hole in the cork.

D—The fourth portion was tightly corked but opened occasionally throughout the year.

These four samples were tested upon dogs at the end of twelve months, with the result that with *A*, no loss of blood-pressure raising power was sustained; this was also true of the percentage of total alkaloids. Of the other samples, that tightly corked (*B*) deteriorated the least (about 35 per cent.), but the strength of all except *A* was less than at the beginning of the experiments. A marked deterioration was noted in *C* and *D*, *D* (tightly corked but opened occasionally, conditions under which the preparation is commonly kept), showing the most marked deterioration (67 per cent.).

These investigations proved that by adopting the ***vacuum method*** (complete exhaustion and exclusion of the air from both the container and its contents and then hermetically sealing the container under vacuum) the rate of deterioration can so be retarded as to make these preparations of stable quality for at least a definite time, thus making it possible not only to supply the physician with preparations of these drugs, which will maintain their "standard strength," but also afford the experimenter a means of preserving standard preparations with which he can compare the strengths of new lots of drugs or their preparations and thus adjust them to standard strength.

[1] "A New and Reliable Method for the Preservation of Ergot Preparations," by Pittenger and Vanderkleed, Jour. A. Ph. A., Aug., 1912, p. 799.

(10) TYPE METHODS

Many methods used for the physiologic standardization of drugs are not new but merely *quantitative* applications of methods used to elucidate the drug's physiologic action. The principal task of the biologic chemist, therefore, is the selecting of the most suitable method for the particular drug under consideration.

The principal type methods available for the physiologic study and standardization of the vegetable drugs are the three following:

1. *Toxic methods* in which guinea-pigs, frogs, or some of the higher animals are used, the value of the drug or preparation depending upon the amount required to cause the death of the animal. Examples: *a*, Reed and Vanderkleed's Guinea-pig Method; *b*, the one-hour frog method; *c*, the twelve-hour frog method; and *d*, Hatcher's cat method for the standardization of the heart tonics and depressants.

2. *The amount of drug or preparation required to produce some specific effect upon the intact animal.* Examples: *a*, Cock's comb method for ergot; *b*, the blood-pressure method for epinephrine, pituitary extract, ergot, digitalis, etc.; *c*, the uterine method for ergot.

3. *The amount required to produce a definite effect on an isolated organ.* Examples: *a*, isolated uterus method for ergot and pituitary extracts; *b*, the perfusion method for the digitalis series.

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CHAPTER II

CARDIAC STIMULANTS AND DEPRESSANTS

The most important drugs included under the above heads are:

(a) Stimulants
- Apocynum.
- Convallaria.
- Digitalis.
- Squills.
- Strophanthus.
- Epinephrine.[1]

(b) Depressants
- Aconite.
- Gelsemium.
- Veratrum.

Stimulants.—The cardiac stimulants are commonly known and referred to as the "*digitalis group*" because of the similarity of their actions to those of digitalis.

The distinguishing feature of this group consists in its power to produce an increased tone of muscular tissue, generally manifested most conspicuously on arterial and cardiac muscle, leading to increased strength and duration of the systole, and to rise of blood-pressure.

This series of drugs possesses a local and a general action. The action on the heart, however, is the most important of all, and is what distinguishes digitalis and its allies from all other substances. This action has been studied most carefully in the frog, and is found to be due to an alteration in the cardiac muscular tissue. On exposing the frog's heart and watching its movements after the injection of digitalis, the muscular action can generally be made out very distinctly (Fig. 1). The heart becomes slower in rhythm, and contracts to smaller dimensions in systole, while it does not dilate so fully in diastole. During systole it is, therefore, whiter than in normal contraction, while during diastole it is less purple than in normal dilatation, owing to its con-

[1] The actions and standardization of epinephrine are treated separately in Chapter III, p. 49.

taining less blood at each period. The slowing can be seen to be due to the heart's remaining contracted longer than usual, while the dilatation is very short and imperfect. Later the apex of the ventricle ceases to dilate during diastole, and remains quite still while the base still dilates after each auricular systole. Or the whole ventricle dilates only once for every two contractions of the auricle, or the two halves of the ventricle may contract alternately so that the blood is thrown from one side to the other. Meanwhile the duration of systole becomes still

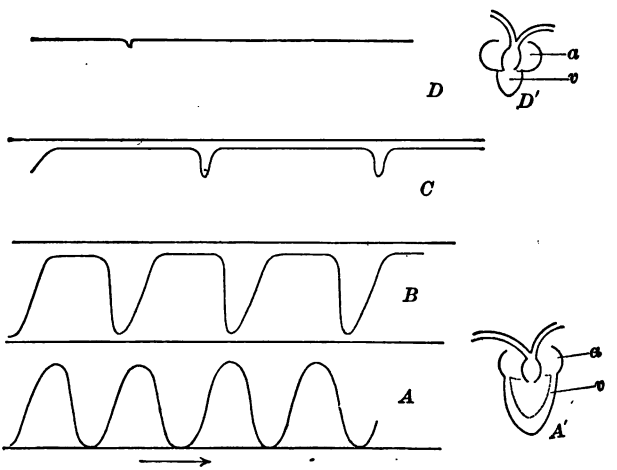

FIG. 1.—Tracing of the movement of the frog's ventricle under digitalis. The lever forms an upward stroke during systole. *A*, normal. *B*, the systole is somewhat more complete and is very prolonged, and the rhythm is correspondingly slow. *C*, the ventricle remains in systole with occasional feeble diastolic movements. *D*, the diastoles of the heart have almost entirely ceased. *A'*, diagram of the heart of the frog in its normal dimensions, *a*, auricle; *v*, ventricle with the aortic bulb rising from it. The dotted line in the ventricle represents the outline in systole, the continuous line the outline in diastole. *D'*, outline of the heart in standstill after digitalis. The ventricle *v* is very much contracted, the auricle *a* distended with blood. (Cushny.)

more prolonged, and the extent of diastolic dilatation diminishes until the ventricle finally ceases to relax, remaining in a position of extreme systole with its cavity obliterated. The auricles come to a standstill also, but they are unable to empty themselves into the contracted ventricle and therefore remain distended with blood. The typical action of digitalis on the muscle of the frog's heart, then, consists in a tendency to increase and prolong contraction, and diminish and shorten diastole (Cushny).

The action of the digitalis series may be divided into three stages:

1. The *therapeutic or first stage* characterized by an acceleration of the heart and a rise in blood-pressure.

2. The *inhibitory or second stage* causing a low blood-pressure from a lessened output of the heart.

3. The *toxic or third stage* characterized by marked irregularities of the heart, during which the blood-pressure rises again from the increased output of the heart and the further contraction of the vessels.

METHOD OF RECORDING THE ACTION OF THE HEART TONICS AND DEPRESSANTS (AND OTHER DRUGS) UPON THE FROG'S HEART

Apparatus Necessary for Experiment.—Small kymograph, iron stand with clamps, frog board, Harvard heart lever; probe, scissors, tweezers, scalpel, pipette, normal salt solution; drug solutions. If a time tracing is desired the experiment will also require a signal magnet, dry cells and an electric clock.

Animals.—Medium size frogs of about 35–40 gm. are best adapted for this purpose.

Preparation for Experiment.—The drum is removed from the kymograph, covered with glazed paper, smoked and replaced. Next the frog board, heart lever, and signal magnet are assembled as shown in Fig. 2. The frog is then *pithed.* This is accomplished as follows:

Wrap the frog in a towel allowing only the head to protrude. The frog is held in the left hand and the head bent slightly forward with the left thumb. If the nail of the right forefinger is passed lightly along the spine the articulation between the skull and the vertebral column can be felt at the point where the cerebrospinal canal has no bony covering. The canal is punctured at this point by a narrow-bladed knife after which the brain is destroyed by inserting a probe at this point and pushing it into the brain cavity, gently moving it from side to side. The point of the probe is now turned and the spinal cord is destroyed in a similar manner. This final stimulation of the nerve cells causes a discharge of motor impulses to the muscles of the body, which give a series of convulsive twitches or contractions. These twitches quickly cease, the body and limbs become toneless and relaxed and the reflexes abolished.

The animal is pinned on a frog board with the abdomen uppermost;

the skin over the abdomen is pinched up and slit to the mouth; the abdominal wall is then divided slightly at one side of the middle line to avoid cutting the anterior vein of the abdomen. By a transverse cut the sternum is then divided; the junction of the anterior abdominal vein with the heart is preserved. The chest girdle is next divided in the middle line, the inner blade of the scissors being kept hard against the sternum to avoid injuring the heart beneath. The divided halves

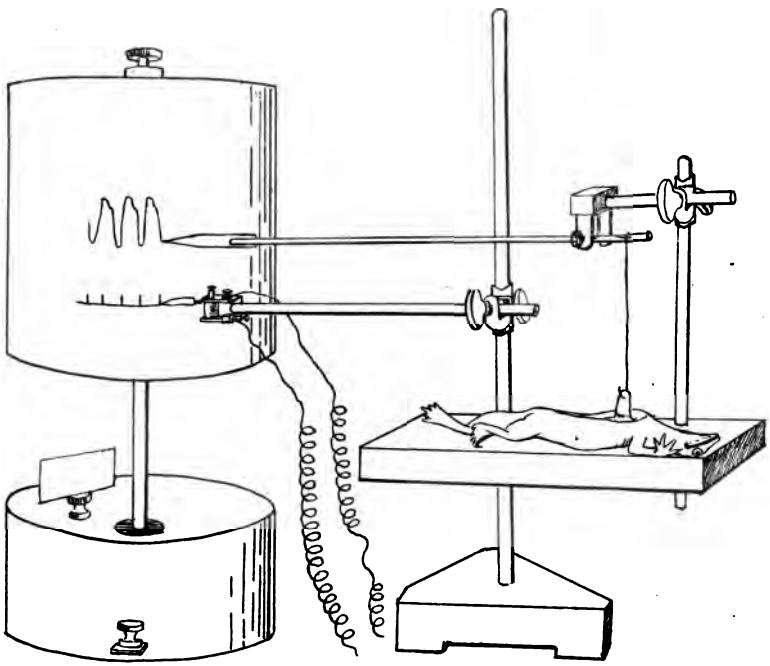

FIG. 2.—Showing arrangement of apparatus for recording the action of the frog's heart in place in the body. (From Greene's Experimental Pharmacology.)

of the pectoral girdle are then pulled widely apart and the heart is found to be enveloped by a thin membrane, the pericardium. Pick up the pericardium with the forceps and slit it open. On the posterior surface of the heart is a slender band of connective tissue, the frænum, which should be divided. Next hook a small sized pin into the tip of the heart, attach to it a thread which is next to be connected with the heart lever. Counterpoise the heart lever and let it record the movements of the heart on the smoked drum.

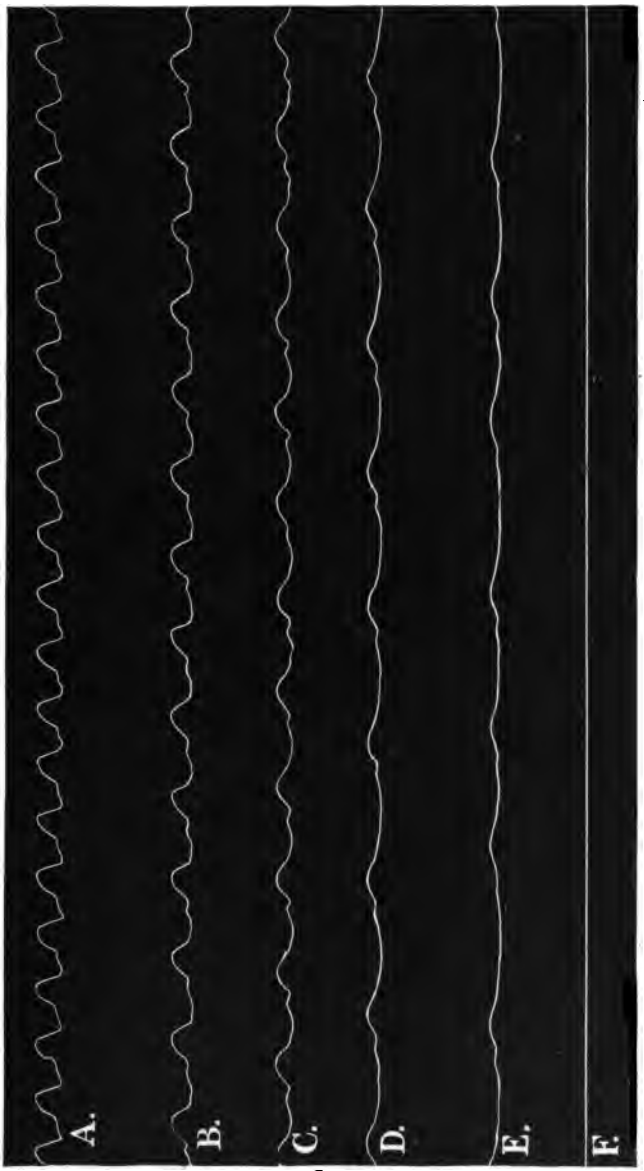

FIG. 3.—Action of digitalis on frog's heart. The lever forms an upwad stroke during systole. *A*, normal; *B*, *C*, *D* and *E* show the action of the drug. Note the gradual lengthening of the systole, also the gradual decrease in the amplitude of the contractions caused by the ventricle relaxing less and less during diastole; *F*, heart tonically contracted in systolic standstill.

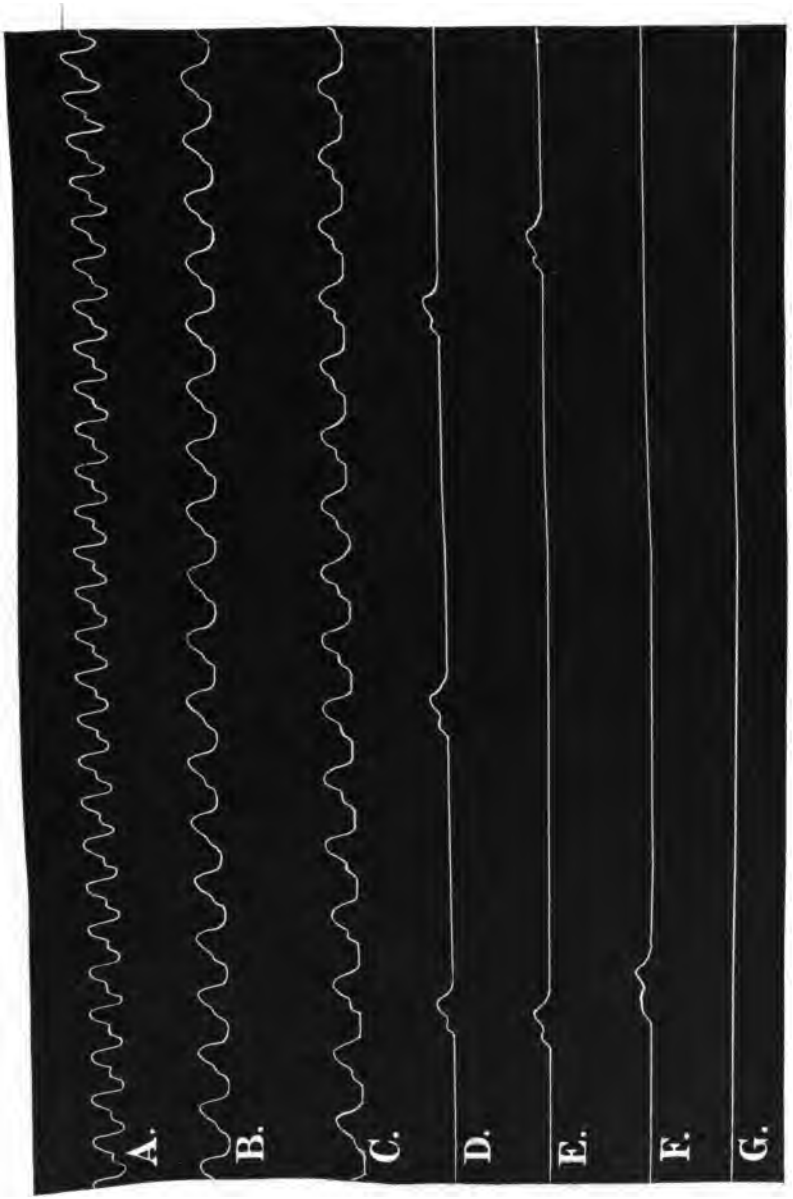

FIG. 4.—Action of aconite on frog's heart. *A*, normal; *B*, *C*, *D*, *E*, *F*, and *G* show the slowing and final stoppage of the heart due to the action of the drug.

Preparation of Solutions.—Normal saline solution for frogs is prepared by dissolving 30 gm. of sodium chloride in 4000 c.c. of distilled water. The *drug solution* is prepared by dissolving the drug in physiological saline. If the preparation to be tested is a tincture or a fluid extract it should be freed from the greater part of alcohol by evaporation on the water bath, and diluted with normal saline solution to the desired volume.

Experiment. *Effect of the Heart Tonics on the Frog's Heart.*—First record the normal movements of the heart, then irrigate with solution of digitalis for about two minutes. Take several tracings one beneath the other until the heart finally comes to standstill in systole. (See Fig. 3.) The heart is slowed by lengthening of the systolic contractions, which are also more powerful. At first the output of the heart is also increased. As the action progresses the systole becomes longer and stronger and the ventricle relaxes less and less during diastole, retaining a remarkably white appearance. The output is lessened, diastolic relaxation is finally abolished altogether, and the heart remains tonically contracted, in systolic standstill.

"In some cases certain other features appear in the frog's heart, for the slow rhythm may be accompanied by a less perfect systole, and instead of the heart ceasing in systole it may come to a temporary standstill in a state of extreme diastolic dilatation. This is due to stimulation of the vagus center in the medulla, and must be carefully distinguished from the action on the cardiac muscle. Not infrequently the two forms occur in combination, or the symptoms of inhibitory actions precede those of the true cardiac change" (Cushny).

Depressants.—The principal action of the members of this group is a lowering of the activity of the heart. There are two ways in which the drug accomplishes this, *i.e.*, 1. by stimulation of the vagus mechanism; 2. by weakening of the cardiac muscle itself. The latter effect may be produced, however, by large doses of almost any drug; therefore, the former is alone useful therapeutically.

Experiment. *Effect of the Depressants upon the Frog's Heart.*—Arrange apparatus as described under "Heart Stimulants" (page 14); connect heart with writing lever and record normal movements; irrigate with 0.1 per cent. solution of aconite in Ringer's solution for about two minutes. Take several tracings one beneath the other until the heart stops. (See Fig. 4.)

It will be noted by Fig. 4 that the frog's heart after preliminary

FIG. 5.—Effect of digitalis on blood-pressure. Drug injected intravenously from *X* to *X*; *A*, normal; *B*, fall in pressure during the injection, caused by the local irritant action on the heart and vessels; *C*, therapeutic stage; *D*, toxic stage.

quickening from stimulation of the accelerator endings and cardiac muscle soon passes into a state characterized by slow pulse and prolonged diastole, produced by stimulation of the inhibitory mechanism.

Effect of Heart Tonics and Depressants upon Blood-pressure. *Apparatus Necessary for Experiment; Animals; Preparation of Experiment; Method of Injecting.*—Same as required for the standardization of epinephrine. (See page 52.)

Experiment. *Effect of Heart Tonics.*—After all preliminary arrangments have been made bring the writing point of the manometer to bear upon the smoked paper of the kymograph. The blood-pressure tracing is then started on a slowly revolving drum. After obtaining a tracing of normal pressure about three inches in length, inject a toxic dose of tincture of digitalis into the saphenous vein; take continuous tracing until heart stops. The best tracings are produced by doses which cause the stoppage of the heart in about fifteen to twenty minutes. (See Fig. 5.)

"In the *first or therapeutic stage* of the action of this series, the rhythm of the heart is changed and the extent of contraction and relaxation of the ventricle and auricle undergo certain modifications. The rhythm of the heart is distinctly slower than before giving the drug, for the inhibitory apparatus is set in activity and the slowing is accordingly due to a pro-

longation of the pause in diastole. The ventricles contract to a smaller size, that is, they empty themselves much more completely than they normally do. It is now universally recognized that the normal ventricle does not empty itself completely; that even at the end of its systole there still remains some blood in its interior. After the action of this group has begun, however, the blood remaining at the end of systole is much less than before. This increased contraction is, like that in the frog's heart, due to action on the cardiac muscle.

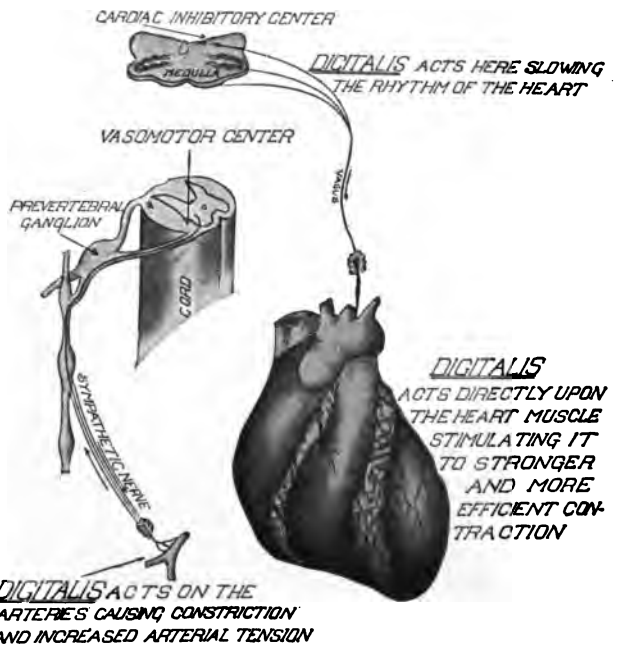

FIG. 6.—Illustration of the action of digitalis on the heart and blood-vessels.

The papillary muscles undergo the same changes as the rest of the ventricular wall, contracting more strongly and more completely than before the administration of the drug.

"In the *second stage* the symptoms are due to excessive inhibitory activity, while the direct cardiac action is less developed. The rhythm of the ventricle, and consequently of the pulse, is very slow and irregular, as is always the case when the inhibitory apparatus is strongly stimulated. During diastole the ventricle dilates more completely

than usual, while its systole varies in strength. If the muscular action is well developed, it continues to empty itself more completely than usual, but very often the inhibition is so powerful that the muscular action is entirely concealed and the systole is weaker and more blood remains at the end of the contraction than before the drug was administered. As a general rule, however, each beat expels more blood than normally, because the heart is engorged before the systole begins; but the rhythm is now so slow that the output per minute and the efficiency of the heart as a pump is less than usual. This is the feature which differentiates the first from the second stage, in which the same factors are present; in the first stage the efficiency of the heart, *i.e.*, the amount of blood expelled per minute, is greater, in the second stage less than before the administration of the drug.

"When very large quantities of any of this series are injected, the *third stage* sets in. It is preceded by the first for a short time, generally by both first and second. In this stage the ventricular rhythm becomes very much accelerated, often beyond the normal, and even beyond that seen after paralysis of the inhibitory nerves. This acceleration has often been supposed to be produced by paralysis of the vagus, but this is not the correct explanation, for stimulation of this nerve sometimes still slows the heart and all this causes dilatation. The acceleration is really due to the drug increasing the irritability of the heart muscle to such an extent that the inhibitory apparatus is no longer able to hold it in check. All the features of the third stage are due to the poison's increasing the irritability of the heart muscle. This leads to acceleration of the beat, and, eventually, through the muscle of one pair of chambers being acted on more than that of the other, to arrhythmia. The extra-systoles are evidently of the same origin, and the final delirium is also to be ascribed to this action" (Cushny).

The following figures demonstrate the various stages of digitalis action on the blood-pressure.

Experiment: *Effect of Depressants.*—Follow directions given under experiment with heart tonics on page 18, substituting tincture of aconite for tincture of digitalis.

Figure 13 shows the effects of a toxic dose of aconite upon blood-pressure. It will be noted that large doses besides stimulating the vagus also exert a direct muscular action and thereby greatly increase the force and especially the rate of the heart, at the same time rendering it extremely arrythmic. As the direct muscular action comes

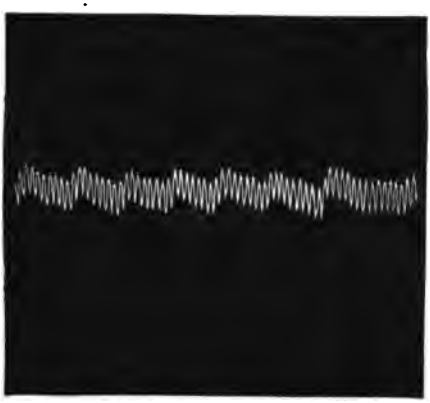

FIG. 7.—Normal blood-pressure tracing. The upward stroke represents the heart systole, down-stroke the commencement of diastole.

FIG. 8.—Action of digitalis, therapeutic stage. Note the increased length of up-stroke (increased strength of contraction); also the increased space between the up-strokes (prolonged diastole).

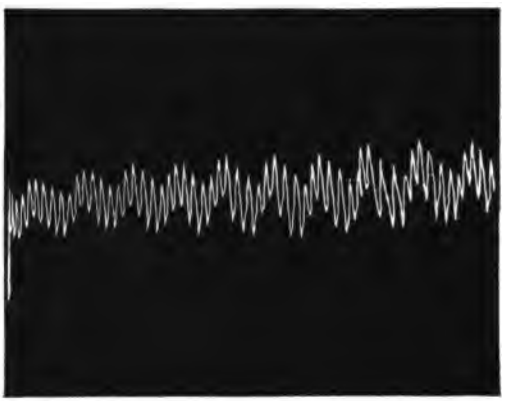

FIG. 9.—Action of digitalis. Combination of therapeutic stage with beginning of intermediate stage; characterized by slight irregularity and exaggerated inhibition from action of the drug upon the inhibitory center in the medulla. Note irregular length and increased space between strokes, showing irregularity of the heart's action preceding the toxic stage.

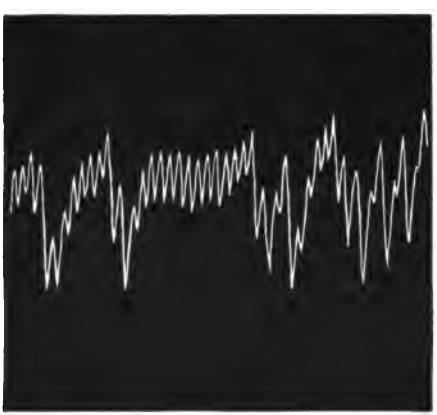

FIG. 10.—Action of digitalis. Extreme inhibition and irregularity.

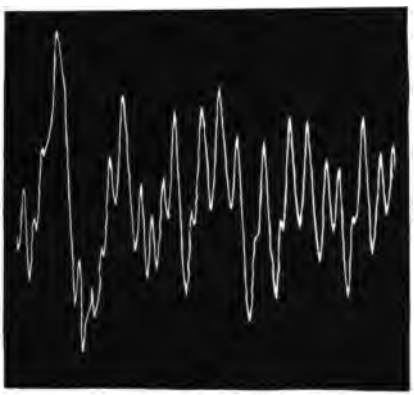

FIG. 11.—Action of digitalis. Marked toxic action.

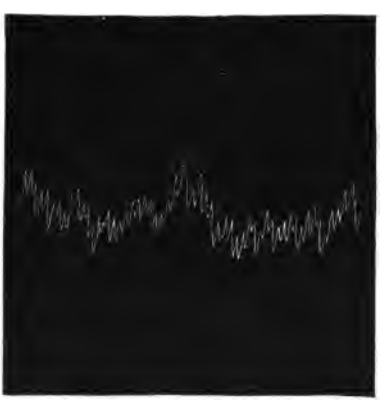

FIG. 12.—Action of digitalis: toxic stage. Delirium cordis, preceding the death of the animal about five minutes. Note the weakness of the contractions and extreme irregularity.

FIG. 13.—Effect of aconite on blood-pressure. Drug injected at arrow. See text.

into play the heart suddenly accelerates from the slow vagus rhythm to one far above normal. Irregularities follow in many different forms after which the heart finally goes quite suddenly into delirium cordis, and stops. The marked acceleration of the heart is due in part to the paralysis of the inhibitory apparatus, but mainly to the direct action on the cardiac muscle. There is, however, no reason to suppose that the direct cardiac action comes into play from the administration of therapeutic doses of the drug. That the acceleration is mainly due to stimulation of the cardiac muscle itself is proven by the fact that the quickening occurs even in the nerve-free heart of the embryonal chick, also by the fact that the drug produces acceleration of the mammalian heart after the nerve endings have previously been paralyzed.

STANDARDIZATION OF CARDIAC STIMULANTS AND DEPRESSANTS

Several methods are available for the quantitative determination of the activity of these drugs and of their preparations. The three principal type methods being:

1. A toxic method, in which guinea-pigs, frogs, and some of the higher animals are used, *e.g.*, Reed and Vanderkleed's guinea-pig method; Hatcher's cat method; Famulener and Lyons's "one-hour" frog method; Houghton's "twelve-hour" frog method, and Focke's frog method.

2. The effect upon blood-pressure.

3. The effect upon the isolated heart of the frog or turtle. (*Example:* Perfusion method.)

1. TOXIC METHODS. (a) **Guinea-pigs. Reed and Vanderkleed's Method.**—This method consists in determining the minimum dose per 250 gm. body-weight of guinea-pig that will prove fatal within twenty-four hours when the drug is subcutaneously injected.

This is perhaps the most convenient and generally serviceable method of valuating the heart tonics and depressants. The guinea-pig is especially well adapted to assay purposes because of its relative slight variation in susceptibility due to age, sex, temperature, seasons, etc., as compared with the large variation found in frogs. The following quotation is taken from the conclusion of a paper based upon the

results of an experiment extending over two years on "Variation in Susceptibility of the Guinea-pig."[1]

"With less than a ten per cent. variation above or below the average, with less than ten per cent. of pigs dying or recovering "out of order" we contend that for all practical purposes, the guinea-pig method affords the simplest and most satisfactory means of standardizing the heart tonic group of drugs, at a very reasonable economic cost, without the necessity of standardizing the test animals, and without need for considering seasonal variations."

Apparatus Necessary for Experiments.—One Hitchens syringe, pipettes graduated in 1/100 c.c., scissors, scales and a set of weights ranging from 1 to 500 gm.

Animals.—Guinea-pigs in good physical condition and weighing from 180 to 400 gm.

Preparation of Experiment.—The guinea-pigs are prepared for the injection by clipping or shaving the hair from about one square inch of the skin over the abdomen, and painting the exposed portion with 5 per cent. tincture of iodine. The pigs are then weighed and records kept.

Preparation of Solutions.—If the preparation to be tested is a tincture or a fluid extract it should be freed from the greater part of alcohol by evaporation on the water bath, and diluted with normal saline solution to the desired volume. Solid and powdered extracts should be dissolved in a sufficient quantity of a suitable menstruum to render the doses accurately measurable. In all cases the preparations should be sufficiently diluted or concentrated, as the case may be, to make the dose injected measure not less than 0.5 c.c. nor more than 4 c.c.

Method of Injecting.—The injections are given subcutaneously, in the abdominal region. Figure 14 illustrates a very simple and handy method.

The Hitchens syringe is especially adapted for this work because it allows no possibility of loss while inserting the needle and may be washed with water without being withdrawn.

[1] Variation in Susceptibility of the Guinea-pig (Continuation of a previously reported study),[2] by Chas. E. Vanderkleed, Phar. D. and Paul S. Pittenger, Phar. D., Read at the Sixty-first Annual Convention of the American Pharmaceutical Association, held at Nashville, Tenn., Aug. 18–23, 1913.

[2] Variation in the Susceptibility of the Guinea-pig to the Heart Tonic Group (Second Paper), by Chas. E. Vanderkleed, Phar. D., and Paul S. Pittenger, Phar. D., Journal of the American Pharmaceutical Association, 11, May, 1913, p. 558.

Method.—Pipette the desired dose of the preparation to be injected, into the side arm (*B*) of the syringe; while holding the syringe with the side arm down, insert the needle as shown below; invert the syringe to allow the liquid to run from *B* to *A;* insert rubber bulb (*C*) into the neck of syringe and inject the liquid by applying pressure to the bulb with the thumb; remove bulb; wash the side arm with about 1 c.c. of water from a "wash bottle"; rotate syringe several times, replace bulb and inject wash-water; massage injected liquid away from the point of injection; withdraw needle.

FIG. 14.—Method of injecting guinea-pigs. *A* and *B*, glass body of Hitchens syringe; *C*, detachable bulb; *D*, needle.

Actual Standardization.—Inject into a series of four guinea-pigs 9/10, 10/10, 11/10, and 12/10 of the standard dose of the preparation to be standardized for each 250 gm. body-weight of guinea-pig. The animals are then placed in cages (see page 120) and allowed to remain for twenty-four hours;[1] when they are examined and a note made of those living and those which are dead.

The results of this preliminary test, in which the range of dosage is quite wide, enables the investigator to form some idea as to the strength

[1] According to the original Reed and Vanderkleed guinea-pig method the results should be recorded *two* hours after the injection of the drug. Later investigations by the author on a series of 1200 guinea-pigs shows that more concordant results can be obtained by using *twenty-four* hours instead of two hours as the time limit.

RESULTS OF EXPERIMENTS ON ANIMALS

Number	Substance	Dose per 250 gm.	Dilution	Dilution per 250 gm.	Description of animal	Weight	Actual dose of dilution	Time	Result and remarks	Date
17683	F. E. Digitalis.	0.08	1–10	0.8	Yellow.....	220	0.7	8.20	Recovered.....	3/12
		0.09	1–10	0.9	Slate.....	260	0.93	8.20	Recovered.....	3/12
		0.1	1–10	1.0	Black.....	235	0.94	8.20	Recovered.....	3/12
		0.11	1–10	1.1	White.....	275	1.21	8.20	Recovered.....	3/12
		0.12	1–10	1.2	Cream.....	210	1.0	8.20	Died.....	3/12
		0.112	1–10	1.12	Blk. and white...	280	1.26	9.00	Recovered.....	3/13
		★0.115	1–10	1.15	Grey.....	245	1.12	9.00	Died.....	3/13
		0.117	1–10	1.17	Yellow and wht..	250	1.17	9.00	Died.....	3/13
		0.112	1–10	1.12	Slate and yellow.	235	1.05	9.30	Recovered.....	3/14
		★0.115	1–10	1.15	Brown.....	240	1.1	9.30	Died.....	3/14

M. L. D. = 0.115 = 86.9% of standard.

FIG. 15.—Sample page from the laboratory book showing the results of an experiment on guinea-pigs to determine the minimum lethal dose of a fluid extract of digitalis.

of the preparation. Basing the dosage upon these results, other series of guinea-pigs are injected with progressively increasing or decreasing doses, as the case may be, still further diminishing the variation between doses, until the smallest amount is found which will prove fatal within twenty-four hours. The probable minimum lethal (toxic) dose of the preparation, unless it deviates considerably from that of the standard, is generally obtained by one or two series of injections. In order to determine whether or not this is the true minimum lethal dose, this result is checked by carefully injecting a new series of four pigs; two with the smallest dose that was found to kill, and two with the largest dose that did not kill. If, however, any of this last series show irregularities, further correction must be made.

Figure 15 shows a convenient method of recording the necessary data pertaining to minimum lethal dose (M.L.D.) experiments. This figure may also be used to demonstrate an assay of fluid extract of digitalis. It will be noted that on the first day (3/12) doses were given ranging from 0.08 to 0.12 c.c. per 250 gm. body-weight of animal; after twenty-four hours the results show that all had recovered except one—that which received a dose of 0.12 c.c. per 250 gm. These results showed that the M.L.D. was between 0.11 and 0.12 c.c. Therefore on the succeeding day (3/13) doses were given between these two, namely, 0.112, 0.115 and 0.117c.c. After twenty-four hours the results show that the pig which had received the dose of 0.112 c.c. recovered, while the two which received 0.115 and 0.117 c.c., respectively, died, thus showing the M.L.D. to be 0.115 c.c. per 250 gm. body-weight. In order to check these results two more pigs were injected, one with 0.112 (the largest dose from which a pig recovered) and the other with 0.115 c.c. (the smallest which had proven fatal). After twenty-four hours it was found that the pig which had received 0.112 c.c. had recovered while the one which received 0.115 c.c. died, thus checking the former results. The M.L.D. for this preparation therefore was 0.115 c.c. per 250 gm. body-weight. After thus determining the M.L.D. the relative strength of the preparation can be calculated by simple proportion as follows:

The M.L.D. of the unknown preparation = 0.115 c.c.
The standard M.L.D. for F.E. digitalis = 0.1 c.c.
(See table of standards on page 30.)
The percentage strength of the unknown would therefore be $0.115 : 0.1 :: 100 : x$ or 86.9 per cent.

In order to express the percentage results it is necessary to adopt for each drug or preparation assayed a standard minimum lethal dose with which the minimum lethal dose of the preparation being tested may be compared. After long experience we have adopted the following provisional standards for our laboratory:

TABLE II

The doses given are per 250 gm. body-weight of guinea-pig.

TINCTURES

Aconite root	0.1
Aconite leaf	0.25
Digitalis	1.0
Gelsemium	2.5
Squills acetic	0.75
Strophanthus	0.025
Veratrum	0.5

FLUID EXTRACTS

Aconite root	0.01
Aconite leaf	0.025
Digitalis	0.1
Gelsemium	0.375
Squills alcoholic	0.25
Squills acetic	0.5
Strophanthus	0.0025
Veratrum	0.05
Convallaria	0.075

SOLID AND POWDERED EXTRACTS

Digitalis	0.025
Gelsemium	0.1
Veratrum	0.015

In actual practice a table arranged as follows is very useful in calculating, from the dose per 250 gm. body-weight of animal the actual dose to be injected. If, for example, a pig weighs 190 gm., a glance at the table shows the factor to be 0.76, which, multiplied by the dose per 250 gm. gives, directly, the actual dose to be injected, thus eliminating the necessity of first dividing the weight of the pig by 250 in order to find the factor.

TABLE III

W. F.	W. F.	W. F.	W. F.	W. F.	W. F.
100–0.40	200–0.80	300–1.20	400–1.60	500–2.00	600–2.40
105–0.42	205–0.82	305–1.22	405–1.62	505–2.02	605–2.42
110–0.44	210–0.84	310–1.24	410–1.64	510–2.04	610–2.44
115–0.46	215–0.86	315–1.26	415–1.66	515–2.06	615–2.46
120–0.48	220–0.88	320–1.28	420–1.68	520–2.08	620–2.48
125–0.50	225–0.90	325–1.30	425–1.70	525–2.10	625–2.50
130–0.52	230–0.92	330–1.32	430–1.72	530–2.12	630–2.52
135–0.54	235–0.94	335–1.34	435–1.74	535–2.14	635–2.54
140–0.56	240–0.96	340–1.36	440–1.76	540–2.16	640–2.56
145–0.58	245–0.98	345–1.38	445–1.78	545–2.18	645–2.58
150–0.60	250–1.00	350–1.40	450–1.80	550–2.20	650–2.60
155–0.62	255–1.02	355–1.42	455–1.82	555–2.22	655–2.62
160–0.64	260–1.04	360–1.44	460–1.84	560–2.24	660–2.64
165–0.66	265–1.06	365–1.46	465–1.86	565–2.26	665–2.66
170–0.68	270–1.08	370–1.48	470–1.88	570–2.28	670–2.68
175–0.70	275–1.10	375–1.50	475–1.90	575–2.30	675–2.70
180–0.72	280–1.12	380–1.52	480–1.92	580–2.32	680–2.72
185–0.74	285–1.14	385–1.54	485–1.94	585–2.34	685–2.74
190–0.76	290–1.16	390–1.56	490–1.96	590–2.36	690–2.76
195–0.78	295–1.18	395–1.58	495–1.98	595–2.38	695–2.78

W = weight of guinea-pig.
F = factor.

(b) **Cats. Hatcher and Brody's Method.**—This method consists in determining the minimum fatal dose per kilogram of cat, when the drug is injected slowly into the femoral vein, the standard chosen for the digitalis group being the cat-unit.

Cat-unit.—The amount of crystalline ouabain which is fatal within about 90 minutes, to a kilogram of cat, when the drug is injected slowly and almost continuously into the femoral vein. A cat-unit is equal to almost precisely 0.1 mg. of crystalline ouabain, or one ten-millionth of the weight of the animal.

Apparatus Necessary for the Experiment.—Accurately graduated syringe or burette; cat-board, two graduated burettes. Operating instruments: scalpels, tweezers, grooved director, hemostat, bulldog clamps, glass seeker, two small glass cannulas, and silk ligatures.

Animals.—Cats of medium size (1.5 to 4 kg.) in good physical condition.

Preparation of Experiment.—After weighing, the animal is completely anesthetized. This is best accomplished by one of the two following methods:—

1. *Ether Anesthesia.*—The animal is placed in an air-tight box or bell-jar, into which is dropped a sponge saturated with ether; the animal is allowed to remain until all voluntary movements cease. It is then removed from the box or jar and fastened upon the cat-board, the anesthetic now being given on cotton covered with a towel, care being taken to cover the whole mouth of the animal.

2. *Morphine Acetone-chloroform Anesthesia* (Edmunds and Cushny).—The animal is placed in a box 35 cm. long, 18 cm. wide, and 18 cm. deep. The box is furnished with a sliding lid. A V-shaped cut is made in the end of the lid and in the corresponding end of the box, so that the animal may be securely clamped in this opening, allowing the head to protrude. The lid is fixed with a nail and then 40 to 60 mg. of morphine are injected with a hypodermic syringe into the skin of the neck. This is followed by 0.3 cm. per kilogram of acetone-chloroform dissolved in alcohol administered by the stomach-tube. As soon as voluntary movements cease the cat is removed from the box and tied upon the board.

Next make an incision two inches long over each saphenous vein at its junction with the femoral vein and sever the tissues just enough to free about one inch of each. A short cannula of small bore, with rubber connection, is then tied into each saphenous vein close to its junction with the femoral vein and the vein clamped off with a small artery clamp.

Preparation of Solutions.—Two solutions are required—*First*, the standard solution is prepared by carefully dissolving 0.1 mg. of crystalline ouabain per kilogram body-weight of animal, in a sufficient quantity of normal saline solution to render it possible to measure accurately aliquot portions of this dose.

Second, the preparation to be standardized should also be carefully diluted with normal saline solution. If a tincture or fluid extract, it should first be freed from part of the alcohol present by evaporation on a water bath.

Methods of Injecting.—Two glass syringes, or, better, graduated burettes, may be used for making the injections. One marked *A*, for the standard solution, is fastened to the rubber connection of the cannula in one saphenous vein, while the other, marked *B*, for

the solution to be standardized, is fastened to the cannula in the other vein. (See Fig. 16.)

Actual Standardization.[1]—"When crystalline ouabain, amorphous strophanthin, or a preparation of strophanthus is to be tested, it is only necessary to inject the solution from a syringe or burette into the femoral vein until the animal begins to show toxic symptoms. The injection is then interrupted, or continued more slowly until the unmistakable signs of approaching death are seen. These signs are so

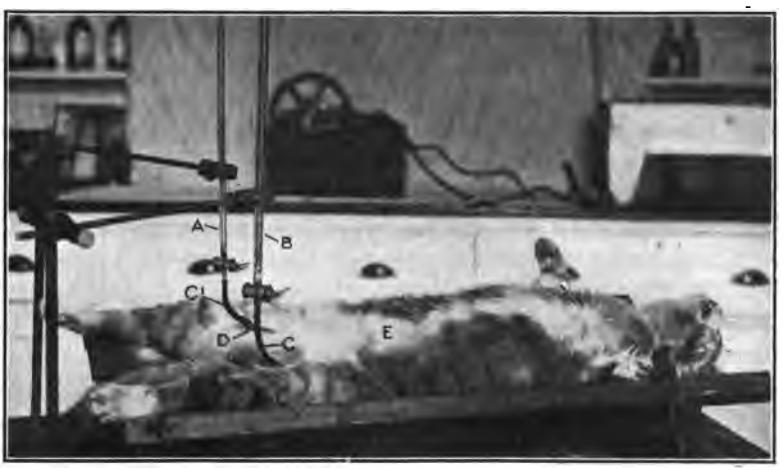

FIG. 16.—Arrangement of apparatus for performing drug assays upon anesthetized cats according to the method of Hatcher and Brody. *a*, burette for injecting the standard ouabain solution; *b*, burette for injecting the unknown preparation; *c* and *c'*, rubber connections; *d*, cannula tied into saphenous vein; *e*, anesthetized cat.

typical that one is rarely mistaken concerning them. They consist in irregularity of the heart, difficult respiration, convulsions, and frequently a peculiar cry, after which recovery is extremely rare. If death does not occur in a few minutes the injection is continued with extreme caution.

"Other members of the digitalis group may be tested in the same manner, but, as a rule, the results will be somewhat too high, and in that case the necessary correction, usually amounting to about 20 per

[1] Hatcher and Brody Method, Am. Jour. Pharm., Aug., 1910, p. 362.

cent., may be made, or the assay may be made more accurately by a modification of the technique.

"Somewhat more uniform results are obtained if about 75 per cent. of the total amount of the digitalis body is injected in the first fifteen minutes and the remainder in the following hour. These results will still be too high, and we have therefore devised a modification of the method of estimating some of the other digitalis bodies which give results that we believe to be nearly as accurate as those obtained with crystalline ouabain itself.

"Just as the analytical chemist may find it desirable to determine the alkalinity of a liquid by adding an excess of acid and titrating back with an alkali, so we have here been able to obtain more accurate results; in some cases when we inject a measured amount of the digitalis body (tincture or infusion of digitalis or digitoxin) in the first period of ten minutes, and after an interval of twenty minutes continue the injection, substituting a solution of crystalline ouabain for that of the digitalis body under examination, until the death of the animal, or until toxic symptoms appear.

"The difference between the amount of crystalline ouabain actually used to complete the assay and the 0.1 mg. per kilogram of animal (the amount which would have been required in the absence of the digitalis body) represents the activity of the digitalis used.

"The following example will illustrate the mode of computing the activity of the digitalis body tested: A tincture representing 70 mg. of digitalis per kilogram of cat was injected into the femoral vein and after twenty minutes the injection of a solution of ouabain was begun. The animal died with the typical symptoms of digitalis poisoning when 0.0142 mg. of crystalline ouabain per kilogram had been injected. The difference between 0.0142 mg. and 0.1 mg. (which would have been required had the ouabain been used alone) is 0.085 mg., or 85.8 per cent., of a cat-unit; hence, 70 mg. of digitalis equals 85.8 per cent. of a cat-unit, and 81.6 mg. of the digitalis equals one cat-unit."

(c) **Frogs. 1. Houghton's "Twelve-hour" Method.**—With this method the strength of the drug is determined by finding the minimum amount per gram body-weight necessary to cause the death of the animal within twelve hours.

Apparatus Necessary for Experiment.—One all-glass syringe or sharp-pointed pipette, graduated into hundredths of a cubic centimeter; frog-boards; tweezers, scissors, scalpel; tank arranged for main-

taining constant temperature; small wire baskets with covers; balance for weighing frogs, and volumetric flasks for making dilutions.

Animals.—The frogs should be healthy, freshly caught, carefully handled, and kept in wet moss until they can be placed in tanks, with running water, in the laboratory. These storage tanks should be kept at a temperature of 10° to 15° C. This is a primary and important essential, as at the ordinary summer heat the frogs die *very* rapidly, while when kept at a temperature of from 10° to 15° C. death rarely occurs.

Another precaution in using frogs as assay animals is that the experiments should be carried out at the same temperature on account of the easy susceptibility of the frog to heat. A temperature of about 20° C. is preferable, because, being about ordinary room temperature, it can easily be maintained. This may best be accomplished at all seasons by means of a simple apparatus consisting of a large galvanized iron tank partly filled with water in which are placed the small cages containing the frogs. The temperature of the tank being raised or lowered merely by heating or cooling the water. (See Fig. 69, page 116).

Since the dose is computed per gram of body-weight, frogs of any size may be used, but it is thought best to use only those of medium weight (20 to 35 gm.) and of nearly uniform size, varying from one another not more than 5 gm.

Any species may be employed, provided both the standard and the unknown preparation are tested upon the same species.

Preparation of Solution.—The preparation to be tested is diluted with a sufficient quantity of normal saline solution to make the doses injected measure about 0.5 c.c.

Method of Injecting.—The injections are made into the abdominal lymph-sac by means of an all-glass syringe or a sharp-pointed pipette.

Standard Preparation.—The standard adopted must possess the physiologic activity of an average preparation. This standard is obtained by mixing equal parts of not less than ten lots of the preparation derived from the same number of lots of crude drugs, which have been determined to be of first-class quality by physical and botanical examinations. Strophanthus is generally employed for this purpose.

Actual Standardization.—The general procedure of injecting several series of animals with progressively increasing or decreasing doses of the preparation to be tested is essentially the same as that given for Reed and Vanderkleed's guinea-pig method (see page 27), differing only in the following points: 1. the results are recorded at the end of twelve

hours instead of twenty-four hours; 2. the minimum lethal dose is expressed in terms of grams per gram body-weight instead of in grams per 250 gm. body-weight; 3. the strength of the preparation being assayed must be compared with the strength of a standard preparation, the two being tested at the same time and under the same conditions.

The following tables showing the results of an assay of tincture of strophanthus will serve as an example of the general procedure.

It is necessary to inject several series of frogs approximately as follows:

TABLE IV

First Series

A. Frogs Injected with Standard

Number of frogs	Weight of frogs	Amount of tincture injected per gram body-weight	Result at end of 12 hours
1	15 gm.	0.0001	Alive
2	16 gm.	0.00015	Alive
3	15 gm.	0.0002	Dead
4	17 gm.	0.00025	Dead

The killing dose is between 0.00015 and 0.0002.

B. Frogs Injected with Tincture to be Assayed

Number of frogs	Weight of frogs	Amount of tincture injected per gram body-weight	Result at end of 12 hours
1	16 gm.	0.0001	Alive
2	15 gm.	0.00015	Alive
3	17 gm.	0.0002	Alive
4	15 gm.	0.00025	Dead

The killing dose is between 0.0002 and 0.00025.

Second Series

A. Standard

Number of frogs	Weight of frogs	Amount of tincture injected per gram body-weight	Result at end of 12 hours
1	15 gm.	0.00015	Alive
2	16 gm.	0.00016	Dead
3	15 gm.	0.00017	Dead
4	17 gm.	0.00018	Dead
5	16 gm.	0.00019	Dead

The killing dose is between 0.00015 and 0.00016.

B. Tincture being Assayed

Number of frogs	Weight of frogs	Amount of tincture injected per gram body-weight	Result at end of 12 hours
1	16 gm.	0.0002	Alive
2	15 gm.	0.00021	Alive
3	15 gm.	0.00022	Dead
4	16 gm.	0.00023	Dead
5	17 gm.	0.00024	Dead

The killing dose is between 0.00021 and 0.00022.

Third Series

A. Standard

Number of frogs	Weight of frogs	Amount of tincture injected per gram body-weight	Result at end of 12 hours
1	15 gm.	0.00016	Alive.
2	16 gm.	0.00016	Dead.
3	16 gm.	0.00016	Dead.
4	17 gm.	0.00016	Dead.
5	16 gm.	0.00016	Alive.

Minimum fatal dose 0.00016.

B. Tincture being Assayed

Number of frogs	Weight of frogs	Amount of tincture injected per gram body-weight	Result at end of 12 hours
1	16 gm.	0.00022	Dead.
2	17 gm.	0.00022	Dead.
3	15 gm.	0.00022	Alive.
4	17 gm.	0.00022	Dead.
5	16 gm.	0.00022	Alive.

Minimum fatal dose 0.00022.

According to the results obtained from the injection of the three series of frogs, 0.00016 c.c. of the standard tincture has the same killing power as 0.00022 c.c. of the tincture being assayed. Hence, the tincture possesses a strength of $\frac{0.00016}{0.00022}=\frac{16}{22}=72+$ per cent. of standard.

A further development of this method of assay led to the adoption of a **"heart tonic unit"** (H.T.U.) as a means of expressing the physiologic values, the number of such units to be derived directly from the minimum lethal dose of the given preparation. A convenient unit is obtained by dividing one by the minimum lethal dose per gram body-weight of frog; or, in other words, the number of units will be the reciprocal of the minimum lethal dose. To illustrate; if the minimum lethal dose of a given drug per gram body-weight of frog is found to be equivalent to 0.01, then the given substance, assuming that it belongs to the group of heart tonics, would contain 100 heart tonic units; $\left(\text{M.L.D.}\ \frac{1}{0.01}\right)=100$ (H.T.U.). This rule can be applied to any of the heart tonics as a means of expressing such values in whole numbers.

Instead of stating the full number of heart tonic units in all cases round numbers can be used, which do not vary more than a given per cent. from the actual number of units (Houghton).[1]

Experience has shown that the figures given in the following list indicate what may be taken as tentative standards for the most important preparations of the digitalis series of heart tonics.

[1] E. M. Houghton: The Lancet, June, 19, 1909.

TABLE V

	M.L.D.	Exact No. of H.T.U. per c.c.	No. of H.T.U. in round numbers per c.c.
Digitalis:			
Fluid extract, U.S.P., 1890	0.0015	66	65
Solid extract	0.0005	200	200
Tincture, U.S.P., 1900	0.015	6	6
Digitalin (Germanic)	0.00005	2000	2000
Squill:			
Fluid extract, U.S.P., 1890	0.0012	83	80
Strophanthus:			
Tincture, U.S.P., 1900	0.000075	1300	1300
Convallaria—Fluid extract:			
Rhizome and roots, U.S.P	0.00025	400	400
Herb	0.00015	666	650
Flowers	0.00009	1111	1100

On account of the variation in the toxicity of the standard and of the different preparations belonging to this series, due to changes in the resistance of frogs, it becomes a matter of considerable importance, as well as convenience to have a table to which one can refer and readily deduce the number of H.T.U. of any preparation after obtaining its M.L.D. A table very well adapted to this purpose is the one prepared by H. C. Hamilton,[1] (pages 41 and 42). "The number of H.T.U. in any given preparation is the reciprocal of ten times the M.L.D. if the frogs are of normal resistance. The resistance of frogs, however, varies greatly, and for this reason the number of H.T.U. per cubic centimeter can evidently not be obtained in so simple a manner.

The factor to be used for adjusting its value is the ratio between the M.L.D. of the standard selected and its average M.L.D. The formula would, therefore, be

$$\frac{1}{10 \times \text{M.L.D. of sample}} \times \frac{\text{M.L.D. of standard}}{\text{Average M.L.D. of standard}} = \text{H.T.U per c.c. or gm.}$$

By means of this formula the correctness of any number in the table may readily be verified.

[1] H. C. Hamilton: Amer. Jour. Pharm., Feb., 1912.

In Table II the numbers in the first horizontal column are the M.L.D. of standard tincture strophanthus, U.S.P., 1890, the range of doses being such as to cover the variation in its toxicity to frogs during the different seasons of the year.

The eight horizontal columns of numbers following this are the M.L.D. for each preparation of the series, with the same range in toxicities as for the tincture mentioned first.

The numbers in the first vertical column beginning with 0.010 are M.L.D. of samples. In this column will be found every possible M.L.D. of members of this series by merely adjusting the decimal point.

All the other numbers in columns A to I inclusive and below the double line are H.T.U. per cubic centimeter or per gram of preparations of the digitalis series of heart tonics, any particular number being the value in terms of heart tonic units of a sample whose M.L.D. is at the head of the horizontal column and the M.L.D. of the standard is in the vertical column which intersects the horizontal at that number.

For example, if a tincture of digitalis has an M.L.D. of 0.02 while that of the standard tincture digitalis is 0.012, it contains 4 H.T.U. per cubic centimeter, this number being found where the columns headed D and 0.020 intersect.

The number representing the H.T.U. of any preparation having an M.L.D. from 0.010 to 0.099 may be found in this way, while those of greater toxicity may be obtained by using a multiple of the number given. For example, if a sample of F.E. Digitalis has the M.L.D. 0.0020 while that of the standard F.E. Digitalis is 0.0012, the sample contains 40 H.T.U. since its toxicity is ten times that used in the first illustration.

It is evident, therefore, that with the data obtained from the assay on frogs one may find in the table the heat tonic units accurately determined for any degree of toxicity.

In a laboratory where samples of every preparation of this series may come in for assay at one time it is inconvenient and, as one can readily see, unnecessary to have an assay of the corresponding standard for each one, since the only object of testing the standard in comparison with the sample is to determine the resistance of the frogs. For this purpose, therefore, in an emergency any one of the preparations might be used as the standard, because a change in the resistance of the frogs would bring about a proportionate change in the M.L.D. of all

TABLE VI

	A	B	C	D	E	F	G	H	I
Tr. Strophanthus, U. S. P. 1890.	.00009	.0001	.00011	.00012	.00013	.00014	**.00015**	.00016	.00017
Tr. Strophanthus, U. S. P. 1900.	.000045	.00005	.000055	.000060	.000065	.00007	**.000075**	.000080	.000085
F. E. Digitalis (70% alcohol)	.0009	.0010	.0011	.0012	.0013	.0014	**.0015**	.0016	.0017
S. E. Digitalis...	.00030	.00033	.00037	.0004	.00043	.00047	**.0005**	.00053	.00057
Tr. Digitalis.....	.009	.010	.011	.012	.013	.014	**.015**	.016	.017
Digitalin.....	.00003	.000033	.000037	.00004	.000043	.000047	**.00005**	.000053	.000057
F. E. Squill, U. S. P. 1890.	.00072	.0008	.00088	.00096	.00104	.00112	**.0012**	.00128	.00136
F. E. Convallaria, U. S. P.	.00015	.00017	.00018	.00020	.00022	.00023	**.00025**	.00027	.00028
Strophanthin.	.0000–006	.0000–0066	.0000–0073	.0000–008	.0000–0086	.0000–0093	**.0000–01**	.0000–0106	.0000–0113
M. L. D. of samples	A	B	C	D	E	F	G	H	I
0.010	6.000	6.667	7.333	8.	8.667	9.333	10.	10.667	11.333
0.011	5.454	6.061	6.667	7.273	7.879	8.485	9.091	9.697	10.303
0.012	5.000	5.555	6.111	6.667	7.222	7.778	8.333	8.889	9.444
0.013	4.615	5.128	5.641	6.154	6.667	7.179	7.692	8.205	8.718
0.014	4.286	4.762	5.238	5.714	6.190	6.667	7.143	7.619	8.095
0.015	4.000	4.444	4.889	5.333	5.778	6.222	6.667	7.111	7.556
0.016	3.750	4.166	4.583	5.000	5.417	5.833	6.250	6.667	7.083
0.017	3.529	3.921	4.314	4.706	5.008	5.490	5.882	6.275	6.667
0.018	3.333	3.704	4.074	4.444	4.814	5.184	5.555	5.926	6.296
0.019	3.158	3.508	3.860	4.211	4.561	4.912	5.263	5.614	5.965
0.020	3.	3.333	3.667	4.000	4.333	4.667	5.	5.333	5.667
0.021	2.857	3.175	3.492	3.810	4.127	4.444	4.762	5.079	5.397
0.022	2.727	3.030	3.333	3.636	3.939	4.242	4.545	4.848	5.151
0.023	2.609	2.899	3.188	3.478	3.768	4.058	4.348	4.638	4.927
0.024	2.500	2.778	3.056	3.333	3.611	3.889	4.167	4.444	4.722
0.025	2.400	2.667	2.933	3.200	3.467	3.733	4.	4.267	4.533
0.026	2.307	2.564	2.820	3.077	3.333	3.589	3.846	4.102	4.359
0.027	2.222	2.469	2.716	2.963	3.210	3.457	3.703	3.951	4.197
0.028	2.143	2.381	2.619	2.857	3.095	3.333	3.572	3.810	4.047
0.029	2.069	2.300	2.529	2.759	2.988	3.218	3.448	3.678	3.908
0.030	2.000	2.222	2.444	2.667	2.889	3.111	3.333	3.555	3.778
0.031	1.935	2.151	2.366	2.581	2.796	3.011	3.226	3.441	3.656
0.032	1.875	2.083	2.292	2.500	2.709	2.917	3.125	3.333	3.541
0.033	1.818	2.020	2.222	2.424	2.626	2.828	3.030	3.232	3.434
00.34	1.765	1.960	2.157	2.353	2.549	2.745	2.941	3.137	3.333
0.035	1.714	1.905	2.095	2.286	2.476	2.667	2.857	3.048	3.238
0.036	1.667	1.852	2.037	2.222	2.407	2.592	2.778	2.963	3.148
0.037	1.621	1.802	1.982	2.162	2.342	2.523	2.703	2.883	3.063
0.038	1.579	1.754	1.930	2.105	2.281	2.456	2.632	2.807	2.982
0.039	1.538	1.710	1.880	2.051	2.222	2.393	2.564	2.735	2.906
0.040	1.500	1.667	1.833	2.000	2.167	2.333	2.500	2.667	2.833
0.041	1.463	1.626	1.789	1.951	2.114	2.276	2.439	2.602	2.764
0.042	1.429	1.587	1.746	1.905	2.064	2.222	2.381	2.540	2.698
0.043	1.396	1.550	1.705	1.860	2.016	2.170	2.326	2.481	2.636
0.044	1.363	1.515	1.667	1.818	1.969	2.121	2.272	2.424	2.575
0.045	1.333	1.481	1.630	1.778	1.926	2.074	2.222	2.370	2.518

TABLE VI.—*Continued*

M. L. D. of Samples	A	B	C	D	E	F	G	H	I
0.046	1.304	1.449	1.594	1.739	1.884	2.029	2.174	2.319	2.463
0.047	1.277	1.418	1.560	1.702	1.844	1.986	2.128	2.270	2.411
0.048	1.250	1.390	1.528	1.667	1.806	1.944	2.083	2.222	2.361
0.049	1.224	1.360	1.497	1.633	1.768	1.905	2.041	2.177	2.313
0.050	1.200	1.333	1.467	1.600	1.733	1.867	2.000	2.133	2.266
0.051	1.176	1.307	1.438	1.569	1.699	1.830	1.961	2.091	2.222
0.052	1.153	1.282	1.410	1.538	1.667	1.795	1.923	2.051	2.179
0.053	1.132	1.258	1.384	1.510	1.635	1.761	1.887	2.013	2.138
0.054	1.111	1.234	1.358	1.481	1.605	1.728	1.851	1.975	2.098
0.055	1.091	1.212	1.333	1.455	1.576	1.697	1.818	1.939	2.060
0.056	1.071	1.191	1.310	1.429	1.548	1.667	1.786	1.905	2.024
0.057	1.053	1.170	1.287	1.404	1.520	1.637	1.754	1.871	1.988
0.058	1.034	1.150	1.264	1.379	1.494	1.609	1.724	1.839	1.954
0.059	1.017	1.130	1.243	1.356	1.469	1.582	1.695	1.808	1.921
0.060	1.	1.111	1.222	1.333	1.444	1.555	1.667	1.778	1.889
0.061	0.984	1.093	1.202	1.311	1.421	1.530	1.639	1.749	1.858
0.062	0.968	1.075	1.183	1.290	1.398	1.505	1.613	1.720	1.828
0.063	0.952	1.058	1.164	1.270	1.376	1.481	1.587	1.693	1.799
0.064	0.938	1.042	1.146	1.250	1.354	1.458	1.562	1.667	1.771
0.065	0.923	1.026	1.128	1.231	1.333	1.436	1.538	1.641	1.744
0.066	0.909	1.010	1.111	1.212	1.313	1.414	1.515	1.616	1.717
0.067	0.896	0.995	1.095	1.194	1.294	1.393	1.492	1.592.	1.691
0.068	0.882	0.980	1.078	1.176	1.274	1.372	1.470	1.568	1.667
0.069	0.869	0.966	1.063	1.159	1.256	1.352	1.449	1.546	1.642
0.070	0.857	0.952	1.048	1.143	1.238	1.333	1.428	1.524	1.619
0.071	0.845	0.939	1.033	1.127	1.221	1.315	1.408	1.502	1.596
0.072	0.833	0.926	1.018	1.111	1.203	1.296	1.388	1.481	1.574
0.073	0.822	0.913	1.005	1.096	1.187	1.278	1.370	1.461	1.552
0.074	0.810	0.901	0.991	1.081	1.171	1.261	1.351	1.441	1.531
0.075	0.800	0.889	0.978	1.067	1.156	1.244	1.333	1.422	1.511
0.076	0.789	0.877	0.965	1.053	1.140	1.228	1.316	1.403	1.491
0.077	0.779	0.866	0.952	1.039	1.125	1.212	1.300	1.385	1.472
0.078	0.770	0.855	0.940	1.026	1.111	1.196	1.282	1.368	1.453
0.079	0.759	0.844	0.928	1.013	1.097	1.181	1.266	1.350	1.435
0.080	0.750	0.833	0.917	1.000	1.083	1.167	1.250	1.333	1.417
0.081	0.741	0.823	0.905	0.988	1.070	1.152	1.235	1.317	1.399
0.082	0.732	0.813	0.894	0.976	1.057	1.138	1.220	1.301	1.382
0.083	0.723	0.803	0.884	0.964	1.044	1.124	1.205	1.285	1.365
0.084	0.714	0.793	0.873	0.952	1.032	1.111	1.190	1.270	1.349
0.085	0.706	0.784	0.863	0.941	1.020	1.098	1.176	1.255	1.333
0.086	0.698	0.775	0.853	0.930	1.008	1.085	1.163	1.240	1.318
0.087	0.690	0.766	0.843	0.920	0.996	1.073	1.149	1.226	1.303
0.088	0.682	0.757	0.833	0.909	0.985	1.060	1.136	1.212	1.288
0.089	0.674	0.749	0.824	0.900	0.974	1.049	1.124	1.198	1.273
0.090	0.667	0.741	0.815	0.889	0.963	1.037	1.111	1.185	1.259
0.091	0.659	0.733	0.806	0.879	0.952	1.026	1.099	1.172	1.245
0.092	0.652	0.725	0.797	0.870	0.942	1.015	1.087	1.160	1.232
0.093	0.645	0.717	0.789	0.860	0.931	1.004	1.075	1.147	1.219
0.094	0.638	0.709	0.780	0.851	0.922	0.993	1.064	1.135	1.206
0.095	0.632	0.702	0.772	0.842	0.912	0.982	1.053	1.123	1.193
0.096	0.625	0.694	0.764	0.833	0.903	0.972	1.042	1.111	1.180
0.097	0.619	0.687	0.756	0.825	0.893	0.962	1.031	1.099	1.168
0.098	0.612	0.680	0.748	0.816	0.884	0.952	1.020	1.088	1.156
0.099	0.606	0.673	0.741	0.808	0.875	0.943	1.010	1.077	1.145

the standards. Whatever standard is adopted, however, should be a product least subject to changes in its activity from any cause. Pure crystalline Kombé Strophanthin is the one which seems to meet all the requirements. This product was finally selected and reported at a meeting of the Philadelphia Section of the American Pharmaceutical Association in March, 1911 (Houghton, American Druggist, July 24, Sept. 11).

The ninth horizontal column of numbers representing M.L.D. of standard preparations of the digitalis series of heart tonics are those for Kombé Strophanthin. These are enclosed between heavy lines. Kombé Strophanthin contains 100,000 H.T.U. per gram, therefore, when this substance is used as the standard the number of H.T.U. in any sample being tested can be calculated by a simpler formula which is obtained by substituting constants in the one previously given and is merely a rearrangement of it. The formula then becomes

$$\frac{100{,}000 \times \text{M.L.D. strophanthin}}{\text{M.L.D. of sample}} \text{ H.T.U. per c.c. or gm.}$$

which can be used at any time in place of the table. The numbers in the table, however, are accurately calculated, and when available are much more convenient than to make the computation in each case."

2. **Famulener and Lyons's "One-hour" Method.**—This is considered by most workers to be the best of the frog methods. It consists in determining the minimum dose of the drug that will cause permanent systole of the frog's ventricle at the end of exactly one hour.

Apparatus Necessary for Experiment.—Same as under Houghton's "twelve-hour" method.

Animals.—In addition to the precautions given under Houghton's "twelve-hour" method, Famulener and Lyons have suggested that, since frogs are markedly influenced by external surroundings, temperature, season, relation of time of injection to time of feeding, etc., it is advisable to guard against the possibility that the frogs used for any particular series of experiments might be above or below the average susceptibility by standardizing each lot before they are used for assay purposes. For this purpose most workers have adopted crystalline ouabain. When a new lot of frogs is received a test is made by injecting one frog with the standard "minimum dose" of ouabain, fixed by previous experiments, to another 10 per cent. more, and to a third 10 per cent. less, than the standard dose. If the result of this experiment shows that the frogs are not of normal susceptibility it is then necessary

to determine the minimum dose for this particular lot of frogs and to correct all succeeding assays accordingly.

Preparation of Solutions.—The method of preparing the solutions for injection is the same as that given for Houghton's "twelve-hour" method, except that the authors of this method advise that in all cases care shall be taken that the solvent is of the same alcoholic strength, as a certain amount of alcohol is necessary, in many cases, to effect solution of the substance injected.

Method of Injecting.—The solution to be tested is accurately measured by means of an all-glass syringe or sharp-pointed pipette. The floor of the mouth under the tongue is then punctured and the contents of the syringe or pipette delivered directly into the anterior lymph-sac of the animal.

Actual Standardization.—Select and carefully weigh a series of four healthy frogs of approximately standard weight (40 gm.). Inject them as described above with 9/10, 10/10, 11/10, and 12/10, respectively, of the standard dose of the preparation being tested for each 40 gm. body-weight of animal. The animals are then placed in cages in the tank (previously regulated to maintain a temperature of 20° C.), and allowed to remain there for about 55 minutes. They are then removed, pithed (both brain and cord), tied to frog-boards, and the thorax laid open so as to expose the heart. If this is already completely paralyzed, the dose has been excessive; if the pulsation still continues, although at a diminished rate, the dose has been insufficient. Basing the dosage upon these results, other series are then injected with progressively increasing or decreasing doses, as the case may be, still further diminishing the variation between doses until the minimum amount of the drug, per 40 gm. body-weight of animal, is found which will just cause paralysis of the heart's action in one hour.

The authors of this method designate their "minimum dose" as the smallest amount of the drug which, in one hour, will produce the following typical condition:[1]

"The apex of the ventricle is paralyzed, but at the base (region of the auriculoventricular system) there is occasionally a very faint contraction, indicated only by a color wave which is noticed quite commonly to have its origin at the left base of the ventricle, passing thence across the ventricle, while the auricles are much distended but con-

[1] Famulener and Lyons, Proceedings of the A. Ph. A., p. 417, 1902.

tinue to pulsate regularly." The activity of the preparation tested is then computed between the "minimum dose" of the preparation tested and the "standard minimum dose" by simple proportion.

Some slight modifications of this method have been adopted by various workers with the idea of securing greater accuracy—the most important of which are: First, that the assays should all be carried out at constant temperature. Second, that the "minimum dose" should be the smallest amount of the drug which, in one hour, will produce the following condition: The heart is paralyzed, but when stimulated by touching it with the end of a pair of tweezers or scissors it will respond with an additional contraction. If the heart will not give this "contraction on stimulation" the dose has been excessive; if it still pulsates the dose has been insufficient.

3. **Focke's Method.**—With this method the heart is exposed *before* the injection of the drug. The strength of the preparation "V" being determined by means of a formula in which the weight of the frog is divided by the dose multiplied by the time, in minutes, required to complete the reaction.

Apparatus Necessary for Experiment.—Same as under Houghton's "twelve-hour" method.

Animals.—Frogs in good physical condition, which have been in captivity not less than three days and weigh between 25 and 30 gm. They are not to be collected before the end of June and should be kept in tanks supplied with water.

Preparation of Experiment.—Six hours before carrying out the actual standardization several frogs of about standard weight (25 to 30 gm.) should be removed from the tank and placed in jars in the laboratory and kept at a temperature not exceeding 17° C.

Preparation of Solutions.—The solution to be injected is prepared by making a 10 per cent. infusion of the drug.

Method of Injecting.—The injections are made, by means of an all-glass syringe or sharp-pointed pipette, into the two leg lymph-sacs.

Actual Standardization.—An unpithed frog is fastened to the board and the heart carefully exposed in the usual manner without causing any loss of blood; 0.25 c.c. to 0.35 c.c. (about one-fortieth of the weight of the frog) of the infusion is then injected into the lymph-sac of each leg. The heart is then watched and the time required to cause systolic stoppage is noted. This is usually from seven to fifteen minutes. If the heart stops in less then seven minutes, the dose has been too large,

while if it continues to pulsate after twenty minutes it has been too small.

Basing the dosage upon the result of this primary experiment, other frogs are injected with larger or smaller doses, as the case may be, until four are found in which the heart has stopped between seven and fifteen minutes. The frogs are then pithed and weighed.

The toxic value of the preparation (V) is then determined by means of the formula

$$V = \frac{P}{d \times t}$$

in which (P) represents the weight of the frog, (d) the amount of the drug injected, and (t) the time in minutes required to complete the reaction.

The relative strength of the preparation tested may then be expressed in percentage by comparing the (V) as determined by the experiment with the standard (V) adopted for that particular drug.

Focke states that the most concordant results are obtained if the experiments are carried out during the months of July, August, and September, but that accurate results for standardization purposes may be obtained at any season of the year if the animals are previously standardized and all subsequent assays corrected accordingly.

2. BLOOD-PRESSURE METHOD. *Apparatus Necessary for Experiment; Animals; Preparation of Experiment; Method of Injecting.*—Same as given under standardization of epinephrin.

Preparation of Solution.—All preparations to be tested should be diluted with normal saline solution to tincture strength, tinctures and fluid extracts first being freed from the greater part of alcohol by evaporation on a water bath.

Actual Standardization.—The blood-pressure tracing is started on a slowly revolving drum. After obtaining a tracing of normal pressure several inches in length the drum is stopped and 1 c.c. of the preparation to be tested is injected into the femoral vein. Another similar injection is given, after allowing from one and one-half to two hours to elapse, in order that the effects of the previous injection may partially pass away. As the action of this class of drugs is prolonged and cumulative, more than two injections should never be given to the same animal, because the first injection always modifies the succeeding injections. Three or four dogs should be used and the average percentage rise of blood-pressure taken as the figure of potency.

The immediate rise produced by subtoxic doses is never marked, in many cases being so slight that a variation of 15 or even 20 per cent. in the size of the dose injected produces no measurable difference in the resultant rise. This method is, therefore, useful only for *roughly* determining the quantitative activity of a preparation, but does not give results sufficiently accurate to permit its being used for standardization purposes.

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CHAPTER III

EPINEPHRINE AND PRODUCTS OF THE SUPRARENAL GLAND

Epinephrine is 1, 2-dihydroxy-4[2] methyl-amino ethyl-4[1]-01 benzene, $C_6H_3(OH)_2(CHOH.CH_2NHCH_3)$ a substance with feeble basic properties, obtained from the suprarenal gland of the sheep or other animal. It is normally secreted by the suprarenal gland into the blood-vessels.

Epinephrine acts peripherally on a variety of structures probably by stimulating the sympathetic nerve endings. Its most important therapeutic action consists in a constriction of the blood-vessels, with consequent high rise in blood-pressure, a slowing of the heart due to stimulation of the vagus center, and a direct stimulant and tonic effect on the heart muscle. The effects of a single dose are very fleeting but can be renewed by a fresh injection. Moderate doses given to animals either by mouth or hypodermically have practically no action; the characteristic effects of the drug are, therefore, best elicited by its injection into the vein, when it stimulates the terminations of the sympathetic nerves arising from the lumbar and dorsal regions of the spinal cord.

The contraction of the vessels due to epinephrine can be shown by applying it to a mucous membrane, when the part becomes pale and anemic from the constriction of the vessels; this is well seen when the drug is applied to the congested conjunctiva. That the contraction of the vessels is the principal cause of the rise of blood-pressure may be easily shown by the fact that the volume of the organs, the venous pressure, and the outflow of blood from the veins are all diminished during the rise of the arterial pressure.

The effect upon the circulation may be demonstrated by the following experiment.

Apparatus Necessary for Experiment; Animals; Preparation of Experiment; Preparation of Solutions; and Method of Injection.—Same as required for the standardization of epinephrine (see page 52).

Experiment.—After all preliminary arrangements have been made bring the writing point of the manometer to bear upon the smoked paper of the kymograph. The blood-pressure tracing is then started on a slowly revolving drum. After obtaining a tracing of normal pressure about 3 in. in length, inject 6 to 10 minims of a 1 to 10,000 solution of epinephrine into the femoral vein; take continuous tracing until the blood-pressure returns to normal. It will be noted from Fig. 17 that immediately after the intravenous injection of epinephrine

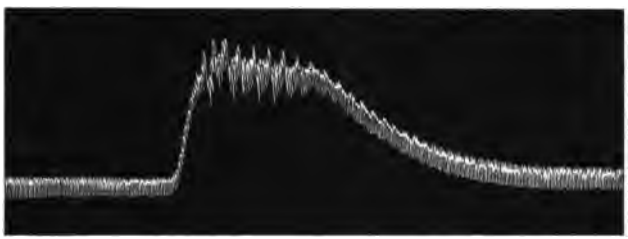

FIG. 17.—Tracing showing effect on blood-pressure of extract of suprarenal gland, which was injected into the femoral vein.

the blood-pressure rises sharply due for the most part to constriction of the vessels of the abdominal cavity; as the pressure approaches its maximum the heart beats are greatly slowed and strengthened. The slowing is obviously due to excitation of the vagus center produced by the increasing blood-pressure. The increase in strength of the contractions is due to stimulation of the terminations of the accelerator nerves in the heart muscle. After the pressure reaches its maximum it is not sustained but quickly returns to normal accompanied by an acceleration, due to the fall in pressure or to the vagus center becoming exhausted, thus allowing the accelerator stimulation again to gain the upper hand.

Stimulation of the Cardiac Muscle.—The effect of suprarenal extracts upon the cardiac muscle may be demonstrated by perfusing the excised mammalian heart with blood containing epinephrine.

Experiment.—For *Apparatus Necessary; Animals; Preparation of Experiment; Preparation of Solutions;* and *Technique employed* see description under "Isolated Mammalian Heart," page 126. First perfuse a cat's heart in the usual way with Loche-blood solution until a normal tracing several inches in length has been obtained, then change to 0.0001 per cent. solution of epinephrine hydrochloride in

FIG. 18.—Action of 0.0001 per cent. epinephrine hydrochloride on the isolated cat's heart. The heart was giving weak contractions just before receiving the drug. Perfused between the arrows with epinephrine. Time in seconds. (From Greene's Experimental Pharmacology.)

Loche-blood solution. Take continuous tracing. It will be noted that immediately after application of the drug the tone is markedly increased; if the heart is beating feebly it often happens that the contractions will increase in amplitude by 200 per cent. or more.

Of the various physiologic actions of the gland above mentioned, the effect upon the blood-pressure presents the best means of physiologic standardization.

BLOOD-PRESSURE METHOD FOR THE STANDARDIZATION OF EPINEPHRINE AND PRODUCTS OF THE SUPRARENAL GLAND

This assay depends upon the characteristic, transitory, quantitative rise in blood-pressure in dogs, produced by the intravenous injection of sub-maximal doses properly diluted.

Apparatus Necessary for Experiment.—A large kymograph with manometer arranged for taking blood-pressure tracings on continuous rolls of smoked paper; accurately graduated all-glass syringe; large and small scalpels; small, sharp-pointed scissors; grooved director; hemostat; two glass seekers; several bulldog clamps; small glass cannulas; silk ligatures; a dog board.

Animals.—Various animals may be employed, the dog, cat, or rabbit; but dogs of medium weight (8 to 14 kilo) give the best results.

Preparation for Experiment.—First completely anesthetize the animal. Any of the volatile anesthetics, such as ether or choloroform, may be employed, but, since it is of great importance that the blood-pressure does not fluctuate from the action of the anesthetic, it is better to employ one of the following methods for this purpose:

1. Inject subcutaneously 0.01 gm. of morphine sulphate for each kilo of body-weight, supplemented by the use of such a quantity of ether as may be necessary to prevent the pain of the operation. After connecting the artery with the manometer the animal is allowed to come from under the influence of the ether. No experiments should be begun until at least ten minutes have intervened after the withdrawal of the ether.

2. Inject subcutaneously 0.01 gm. of morphine sulphate per kilo body-weight of animals, and 45 to 60 minutes later give by mouth 1.5 to 2 gm. of acetone chloroform (1.5 gm. for animals weighing 6 to 7 kilos; 2 gm. for those weighing 10 to 12 kilos, and intermediate weights

accordingly). The acetone chloroform is prepared for administration by shaking it with 4 c.c. of alcohol until dissolved and then adding 4 c.c. of water and again shaking.

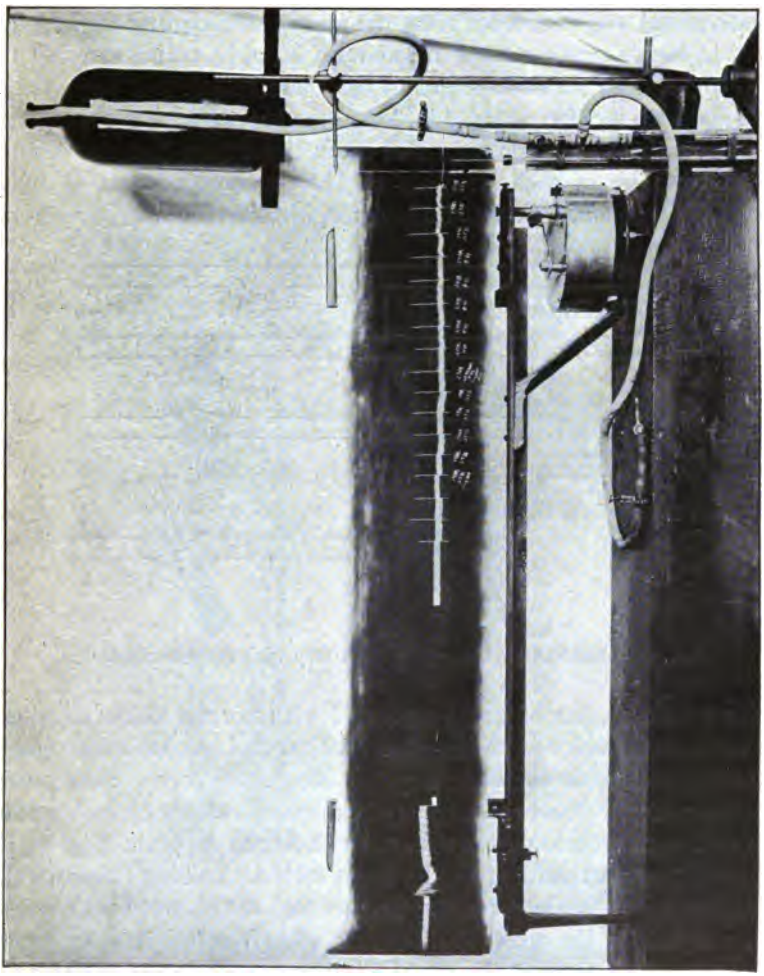

FIG. 19.—Arrangement of apparatus for taking blood-pressure tracing.

The latter method is especially valuable for this work, as it is easily administered and under its influence the blood-pressure and heart-action remain practically constant for hours. I find, however, in many cases, that the animal does not react in such a way as to give concordant

results immediately after the administration of this anesthetic, and therefore advise the following procedure:

3. Administer the anesthetic as set forth above; wait until all voluntary movements have ceased; clip hair from the throat; make an incision about 2 1/2 in. long; sever the tissues surrounding the carotid artery in such a manner as to free about 3 in. of it, taking care not to injure the vagus, which is found in the same sheath (see page 115). Next make an incision about 2 in. long over the saphenous vein at its junction with the femoral vein and sever the tissues just enough to free about 1 in. of each (see page 140); tie a short cannula of small bore in the saphenous, close to its junction with the femoral; cover both

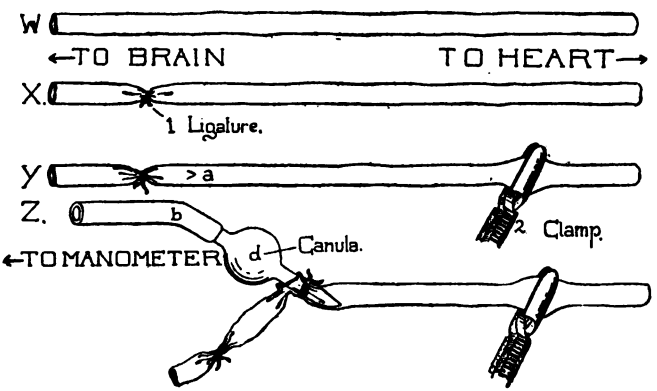

FIG. 20.—Method of connecting the artery with the manometer.

incisions with a piece of gauze saturated with normal saline solution. *The animal is then left in this condition for one and one-half to two hours so that the effects of the anesthetic may partially pass off;* remove the gauze from the neck; lift the exposed part of the carotid artery by means of a glass seeker; tie off that part of the artery leading to the brain (Fig. 20, 1) and close that part leading to the heart with a "bulldog" clamp (2), leaving at least 2 in. between the ligature and the clamp; snip a small V-shaped hole in the artery (*a*), about 1/4 in. from the ligature, with sharp-pointed scissors; make sure that the connecting tube (*b*) and cannula (*d*) are free from air; insert cannula in hole (*a*) and tie the artery fast by means of another ligature (Fig 20, *Z*). Open *C* (Fig. 21) and *E* in order to fill the portion of the artery between the cannula and the bulldog clamp with the magnesium sulphate solution. This keeps the

blood from entering the cannula and thus prevents clotting; close *E;* close *C;* remove clamp from artery; slowly open *C* until the floater *F*

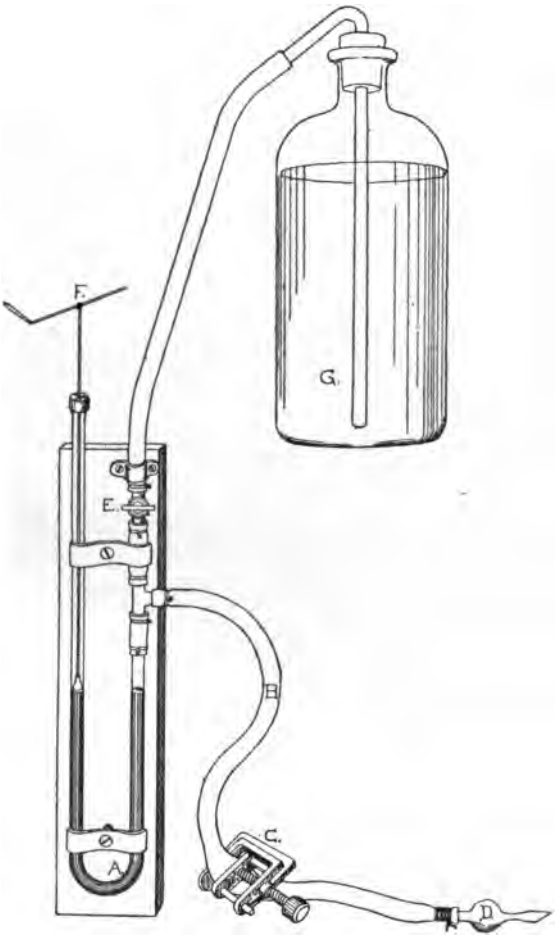

FIG. 21.—Mercurial manometer. *A*, U-tube partially filled with mercury; *B*, connecting tube; *C*, screw-clamp; *D*, cannula; *E*, stopcock; *F*, floater with writing point; *G*, pressure-bottle containing half-saturated magnesium sulphate, or sodium carbonate, solution.

makes an excursion of about 2/8 to 3/8 in. It should then be made to write on a drum which will revolve slowly—about 1 1/2 to 2 in. per minute.

Preparation of Solutions.—The standard solution is prepared by dissolving 1 part of pure epinephrine hydrochloride in 100,000 parts of normal saline solution.

The preparation to be standardized should be carefully diluted with normal saline solution to the same strength, as nearly as may be estimated, of that of the standard used, or of such strength as may be readily diluted in case the primary injection is found to produce too marked a rise in the blood-pressure.

Method of Injecting.—The injections may be made either in the jugular or the femoral vein. The latter is preferable because it is located farther from the heart, thus giving the preparation injected an opportunity to diffuse more thoroughly with the blood before reaching the heart.

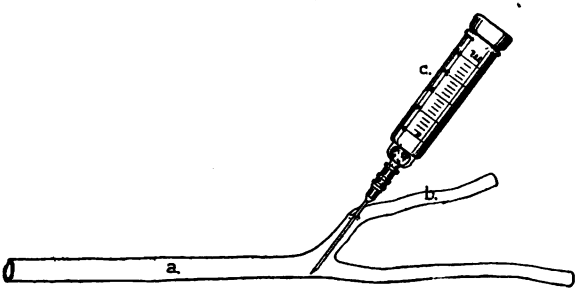

FIG. 22.—Method of injecting. (*a*) Femoral vein. (*b*) Saphenous vein. (*c*) All-glass syringe.

The saphenous vein is lifted and held with a pair of tweezers while the needle of the all-glass syringe is inserted far enough through the cannula in the saphenous vein to allow the point to project into the femoral vein (Fig. 22). After injecting the preparation withdraw the needle and quickly clamp saphenous vein with a bulldog clamp.

The advantage of this method is that, although clamping off the saphenous vein after withdrawing the needle causes clotting, the preparation injected is carried to the heart by means of the main current of blood in the femoral vein.

Another convenient method is to tie a cannula of small bore, with rubber connection, into each saphenous vein close to its junction with the femoral vein. Two graduated burettes may then be used for making the injections. One marked *A*, for the standard solution, is fastened to the rubber connection of the cannula in one saphenous vein,

while the other marked *B*, for the solution to be standardized is fastened to the cannula in the other vein. (See Fig. 16, page 33.)

Actual Standardization. (*a*) *Determination of the Proper Dose of the Standard Solution.*—The blood-pressure tracing is started on a slowly revolving drum. After obtaining a tracing of normal pressure, about 3 in. in length, the drum is stopped. Inject the standard solution in a dose of 0.15 c.c. per kilo. The rise in the blood-pressure should be about 40 to 60 mm. of mercury (equivalent to 20 to 30 mm. rise of the writing-point of the floater in a U-shaped manometer), and it should be submaximal. To determine the latter, a second injection of 0.175 c.c. per kilo should be made after allowing the drum to revolve about 1 in., which should show a higher rise. If the dose of 0.15 c.c.

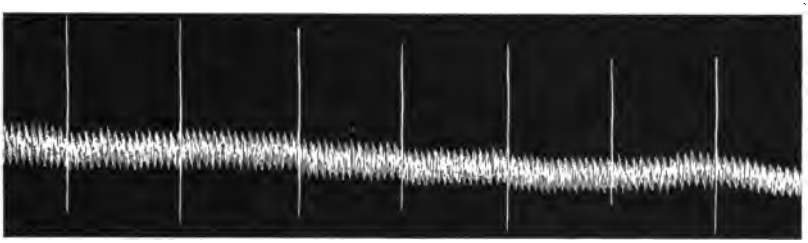

FIG. 23.—Abbreviated tracing showing the method of recording results during an assay of epinephrine.

per kilo does not give a submaximal rise of at least 40 mm., or if it gives a maximal rise, the dose should be increased or reduced, respectively, until the standard effect is obtained, and this dose should be considered the "standard dose." The injections should be made at about the same rate, and an interval of at least three minutes should elapse after the blood-pressure has returned to normal before another injection is made.

The "standard dose" should be large enough to cause *almost* the maximum rise, because the larger the amounts of active principle compared the more accurate the results. It is well known that the stronger the stimulus the more nearly in accord are the results obtained.

(*b*) *Comparison of the Unknown with the Standard Solution.*—A "standard dose" of the unknown solution is injected and the rise of pressure compared with that produced by the "standard dose" of the standard solution. If the difference is very great the unknown solution is strengthened or diluted. The size of the injections is then in-

creased or decreased until that dose of the unknown solution is found which will cause the same rise of pressure as that caused by the "standard dose" of the standard solution. Occasional injections of the standard solution should be made to insure constancy in the reaction of the animal. Final equality is tested by injecting alternately the standard and unknown solutions until the average rise of several consecutive injections is practically equal.

Complete and Abbreviated Tracings.—Two kinds of tracings may be obtained—complete ones (Fig. 17)—when the drum is kept in constant motion, and abbreviated ones (Fig. 23), when the drum remains at rest until the reaction is complete. Abbreviated tracings which give only the maximum blood-pressure obtained from each injection are usually sufficient.

In measuring the tracings it must be remembered that the real rise in blood-pressure is *twice* that which is recorded, since there are two sides to the U-tube and the needle only moves through a space that represents one-half of the difference of level between the mercury in the two sides.

It is understood that no calculations are to be made from relative size of rises caused by similar doses, but always from similar rises, the relation being determined by the size of the dose. More accurate results are obtained if the systolic pressure is alone considered than if the average pressure half-way between systole and diastole is taken for the measurement.

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CHAPTER IV

ERGOT

Ergot is the sclerotium of Claviceps purpurea replacing the grain of the rye (secale cereale). It is of great importance in therapeutics and also in toxicology, as wide-spread epidemics of disease have resulted from eating bread made of rye which has been infected with the fungus.

The chemistry of ergot has been the subject of a large number of investigations, which have been attended with little success until within the last few years. The work of Barger, Dale and their co-workers however, permits us to speak with tolerable certainty of three substances:

1. **Ergotinine,** $C_{35}H_{39}O_5N_5$, is almost inert, but its hydrate, ergotoxine, $C_{35}H_{41}O_6N_5$, has a powerful action on the tissues.

2. **Tyramine or hydroxyphenylethylamine,** $OH.C_6H_4.CH_2CH_2NH_2$ has an important stimulating effect on the heart causing an increase in both the strength and the rate, resulting in a rise in blood-pressure.

3. **Isoamylamine,** $(CH_3)_2CHCH_2CH_2NH_2$, is present in amounts too small to influence the general action of the drug.

In practical medicine ergot is generally administered either in the form of the fluid, solid or powdered extract, and we will, therefore, treat only of the *action of the drug as a whole.*

Ergot is very readily absorbed and on reaching the blood exerts its specific effects on non-striated muscle, directly or indirectly, throughout the body. Its various actions may be summarized as follows.

SUMMARY OF ACTIONS OF ERGOT.[1]

"1. *Stimulation of unstriped muscle,* partly central, but mainly peripheral, the action being exerted in the ganglionic cells or preganglionic endings. This in turn produces:

"2. *Contractions of the uterus,* especially when pregnant (leading to

[1] Sollmann: Text-book of Pharmacology.

abortion); these are intermittent with small doses, tonic and persistent with large doses.

"3. *Vasoconstriction*, differing in extent in different areas, especially powerful in the pulmonary vessels.

"4. With large doses, and in susceptible animals, this leads to *gangrene*, especially in peripherally situated organs.

"5. When rapidly injected, a primary depression and secondary stimulation of the *cardiac muscle*.

"6. *Vomiting* and *increased peristalsis*.

"7. The changes of the circulation leads to affection of the *central nervous system*. These are necessarily variable.

"8. Large doses *paralyze the vasoconstictor endings*."

The following experiments demonstrate the principal actions of the drug.

Experiment. (a) **Effect of Ergot on the Blood-pressure.**—Prepare the animal according to directions given under Epinephrine Standardi-

FIG. 24.—Effect of ergot on the blood-pressure.

zation (see page 52); take tracing of normal pressure about 3 in. in length; inject about 0.08 c.c. per kilo of fluid extract of ergot and take continuous tracing until pressure returns to normal.

An intravenous injection of an active preparation of ergot is immediately followed by an abrupt rise in blood-pressure, either with or without a primary fall. The primary fall in pressure is probably due to such impurities as choline; therefore the better the drug the less the fall. The primary fall of pressure is not seen if the drug is injected subcutaneously. The rise in pressure is to be ascribed to stimulation of the constrictor nerve terminations in the vessel walls and is strictly analogous to that observed under epinephrine. (See page 50.) The sharp rise in pressure is followed in a few minutes by a slight fall, the

pressure still remaining, however, if the dose has not been too large, well above normal. If the dose has been too large it produces toxic effects which cause the rise to give way to a fall which carries the pressure below the normal. If the dose be very large and the fall of pressure is not recovered from, progressive paralysis of the vasomotor apparatus and heart occurs.

Experiment. (b) **Effect of Ergot on the Heart.**—For apparatus necessary, animals, preparation of experiment, preparation of solutions, and technique employed see description under "Isolated Mammalian Heart," page 126. First perfuse heart in the usual manner with Locke-blood solution until a normal tracing several inches in length has been obtained; then change to the drug solution. Allow it to act for about

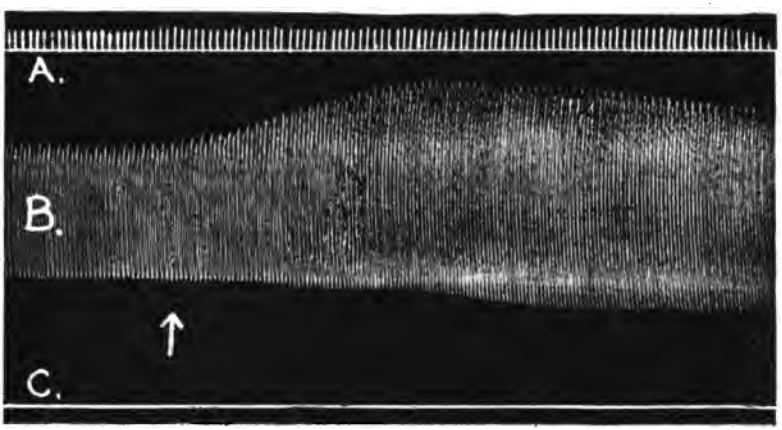

FIG. 25.—Action of ergot on the isolated heart. *A*, time in seconds; *B*, upstroke represents systole; *C*, abscissa. Drug was added at arrow. Method of Langendorff.

five minutes. Take a continuous record. It will be noted that the heart is decidedly and directly influenced by the ergot—it beats more vigorously, its systole is more complete, and its output is considerably increased. This cardiac effect must contribute to the rise of blood-pressure. It is not yet determined whether this change in the heart is due to direct action on the muscle or to a stimulation of the accelerator myoneural junction. Slowing of the heart is frequently seen after an injection of ergot, and this it is claimed arises from stimulation of the vagus center by the high blood-pressure and not from the direct action of the drug. This slowing is sometimes so marked that it par-

tially conceals the effect of the vaso-constriction on the blood-pressure tracing.

Experiment. (c) **Effect of Ergot on the Uterus.**—For apparatus necessary, animals, preparation of experiments, preparation of solutions, method of injecting, and technique employed, see "Isolated Uterus Method," page 73. After all preliminary arrangements are

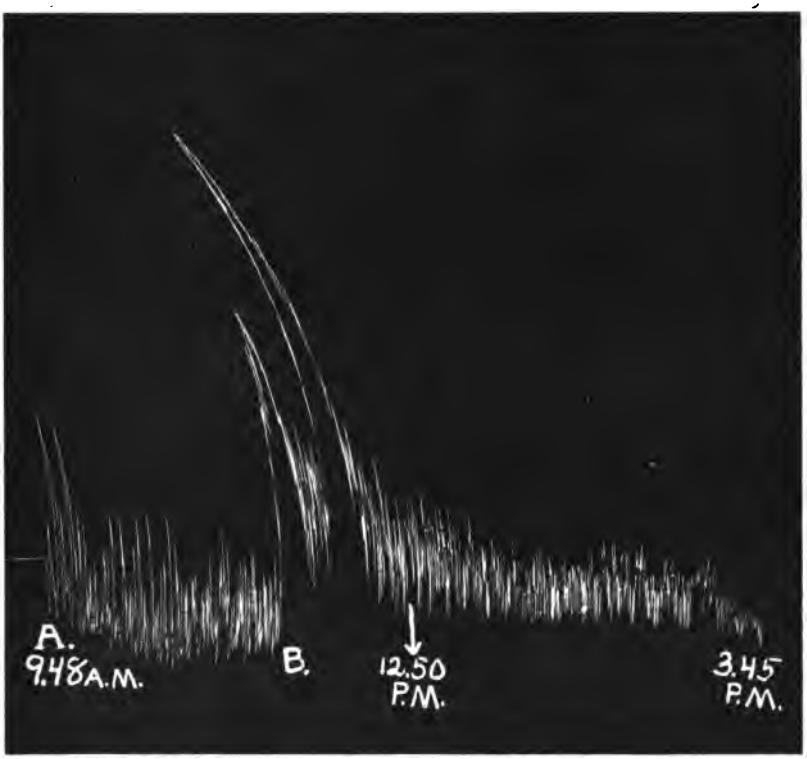

FIG. 26.—Effect of ergot upon the movements of an isolated uterus. Contraction moves the lever upward. *A* to *B* = normal contractions. At *B*, 0.5 c.c., of fluid extract ergot was added.

completed and the writing lever is brought in contact with a very slowly revolving smoked drum a normal tracing should be taken. A dose of about 0.5 c.c. of F.E. ergot should then be added to the Ringer's solution in which the uterus is suspended and a continuous tracing taken. If the preparation given contains only a small amount of activ-

ity it will merely accelerate and strengthen the small rhythmic contractions; on the other hand, should it be a potent extract it will produce a powerful contraction followed by a slow relaxation. (See Fig. 26.) This action is the most important effect of ergot as this drug is mostly em-

FIG. 27.—Demonstrates the two motions of the uterus—the small rhythmic contractions and the tonic contractions.

ployed on account of its effects on the uterus, which effects are primarily produced by its stimulation of the motor myoneural junction of the hypogastric nerves, while the inhibitory nerves are less strongly affected.

There are two motions to the uterus:[1] the small rhythmic

[1] Stewart and Pittenger: "The Application of Some Muscular Tissues Adapted to Physiologic Standardization," Monthly Cyclopedia and Medical Bulletin Sept., 1913.

contractions and the tonic contractions. Ergot in full medicinal doses exerts its influence not by materially increasing the normal pains of labor, but by causing a tetanic, tonic, unyielding uterine spasm which drives all before it. In very small doses it may assist the normal contractions without causing them to become tetanic. It is said that after the administration of small therapeutic doses the tonus alone is increased and the movements of the uterus are not excited; but it is certain that *active* ergot in moderately large doses increases both the peristaltic movements and the tonus.

Figure 27 demonstrates the two motions of the uterus. It will be noted that the small rhythmic contractions are superimposed upon the curves produced by the tonic contractions. In order to show this point more clearly the drum was allowed to revolve at the rate of one revolution per hour, or ten times as fast as in Fig. 26.

STANDARDIZATION OF ERGOT

There are three principal methods available for the physiologic standardization of ergot:—

1. The blood-pressure method.
2. The cock's-comb method.
3. The uterine method—(a) *in situ;* (b) isolated.

1. **Blood-pressure Method.**—This is perhaps the most convenient and generally serviceable test. The technique involved is comparatively easy and, unlike in the cock's-comb method, the effects may be graphically portrayed and accurately measured, thus giving a definite quantitative standard. This method also has a great advantage over the other methods in that *the assay does not require nor depend upon the keeping of a standard preparation which may deteriorate.*

Apparatus Necessary for Experiment; Animals; Preparation of Experiment.—Same as required for the standardization of epinephrine. (See page 52.)

Preparation of Solutions.—All preparations to be tested should be carefully diluted until 1 c.c. of the dilution represents 1 gm. of drug.

Method of Injecting.—Same as that given under Epinephrine Standardization.

Actual Standardization.—The blood-pressure tracing is started on a slowly revolving drum. After obtaining a tracing of normal pressure about 3 in. in length, the drum is stopped. An injection of 0.04 c.c. (gm. of drug) per kilo of the preparation to be tested is then given and

the blood-pressure observed for ten or fifteen minutes. In most cases the injection is followed by a rapid primary fall in pressure, succeeded by an almost equally rapid rise to normal or above—dependent on the strength of the preparation. In order to facilitate the measuring of the myograms, the drum is made to revolve a short distance as follows:

1. When the point is reached where the blood-pressure is about to rise after its primary fall. Fig. 28 (A.)
2. Five minutes after the injection. Fig. 28 (5).
3. Ten minutes after the injection. Fig. 28 (10).
4. Fifteen minutes after the injection. Fig. 28 (15).

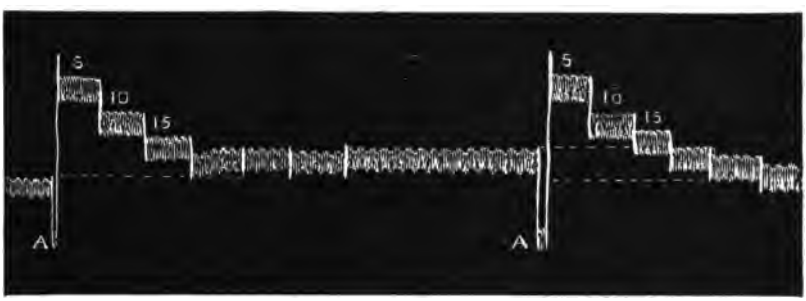

FIG. 28.—Abbreviated Tracing showing method of recording results during an ergot assay. The Figs. 5, 10 and 15 represent the number of minutes after the injection.

If the first injection causes a fall in pressure of more than 35 mm., or a rise of more than 48 mm., the dose should be reduced to 0.02 c.c. per kilo. If the fall is less than 24 mm. and the rise less than 24 mm. the dose should be doubled; otherwise, the same dose is repeated.

Three or four injections are given to one animal, allowing from 60 to 90 minutes to elapse between each injection in order to allow the effects of the previous injection to pass off.

Use of Several Animals.—Three or four injections of the same preparation should be made into each of three or more dogs and the average rise in pressure taken as the figure of potency.

In order to express the activity of the preparation in percentages it is necessary to adopt some provisional standard with which to compare the unknown preparation. After much experience we have adopted the following standard for our laboratory:

"0.08 c.c. of the fluid extract per kilo should cause a rise of blood-pressure of 30 mm."

An objection to this method is that mongrel dogs, such as run at large in the city, differ considerably in their reaction, but, by using two or three dogs for each preparation of ergot, this source of error is considerably reduced.

Another objection to this method has been the lack of proof as to whether or not the action of ergot upon the circulation parallels the action upon the uterus. This is an important factor since the blood-pressure method is rather extensively used for standardization purposes, its employment being supported by statements that the characteristic effect of ergot is a stimulation of all unstriped muscle tissue of the body, and that the changes in the circulation, in the intestines and in the uterus are but a part of one general action. The employment of this method has further been supported by the fact that all the substances which have been suggested by various workers as the active principles of ergot have produced stimulation of the blood-vessels as well as of the uterus. Recent investigations,[1] however, tend to prove that a parallelism does exist between these two actions. If further observations substantiate these results the problem of ergot assay will be considerably simplified.

Among the various methods employed for physiologic standardization, blood-pressure tests consume a comparatively great amount of time. This is especially the case with the blood-pressure method for ergot, as it is necessary to check the results on two or three dogs, and, due to acculative action, it is also necessary to allow from one to one and one-half hours to elapse between injections. With the usual method of using one manometer and kymograph (see Fig. 19, page 53) only one animal can be used at a time, and it therefore requires the greater part of two days to assay one sample of ergot in duplicate, unless several kymographs and manometers are employed. Therefore, in laboratories where considerable numbers of ergot assays are handled and in those where economy of space is essential, it is advisable to employ the following **"Improved Form of Kymograph",**[2] with which it is possible for one man to run blood-pressure tests on four animals at the same time, and record all the tracings on one kymograph without

[1] "A New Uterus-contracting Method of Testing Ergot, with Comparison with the Blood-pressure Method," by Paul S. Pittenger, Phar. D., and Chas. E. Vanderkleed, Phar. D., read at the Sixty-first Annual Convention of the American Pharmaceutical Ass'n., held at Nashville, Tenn., Aug. 18–23, 1913.

[2] "An Improved Form of Kymograph," by Paul S. Pittenger, Phar. D., Journal of the American Pharmaceutical Association, Dec., 1913, p, 1498.

their interfering with each other. This enables one operator to assay at one time with one kymograph two samples of ergot in duplicate, or, he can assay at the same time one sample of ergot in duplicate and one sample of adrenal extract.

The following cut shows the arrangement of the apparatus:

Description of Method of Employing Apparatus.—The animals are prepared for blood-pressure experiments as given under epinephrine standardization. (See page 52.)

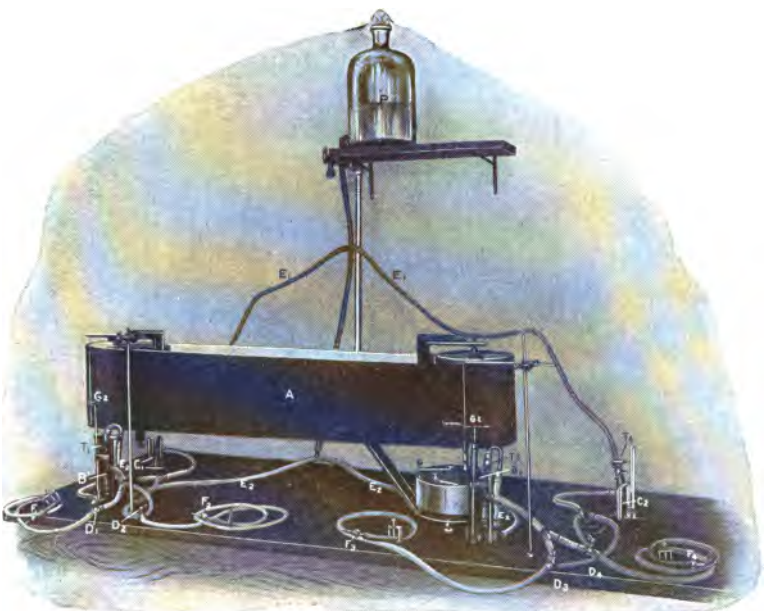

FIG. 29.—Kymograph arranged for making blood-pressure tests on four animals at one time. A, long paper kymograph; B_1 and B_2, manometers with writing points; G_1 and G_2, writing points; C_1 and C_2, dummy manometers, without writing points; D_1, D_2, D_3 and D_4, three-way stopcocks; E_1, tubes used for securing pressure in manometers C_1 and C_2; E_2, tubes used for securing pressure in dummy manometers B_1 and B_2; F_1, F_2, F_3 and F_4, cannulæ; H_1, H_2, H_3 and H_4, connecting tubes; and T_1, T_2, T_3 and T_4, stopcocks.

Each of the four cannulæ (F_1, F_2, F_3 and F_4) is then tied into the carotid artery of a dog. Pressure is obtained within the various tubes from the pressure bottle (P) by opening the cocks T_1, T_2, T_3 and T_4 (T_2 invisible). It will be noted from Fig. 29 that each connecting tube H_1, H_2, H_3 and H_4, terminates in a three-way stopcock which enables

the operator to connect it with either a manometer which writes on the smoked drum, or with a "dummy" manometer.

To assay two samples of ergot it is merely necessary to use two dogs on one end of the kymograph for one sample and two on the other end for the other sample. The three-way stopcocks are arranged in such a manner that one dog on each end records its pulsations upon the revolving drum while the other pulsates against a "dummy" manometer. Inject the proper dose of fluid extract of ergot into the dog which is recording its blood-pressure on the right-hand side of the kymograph;

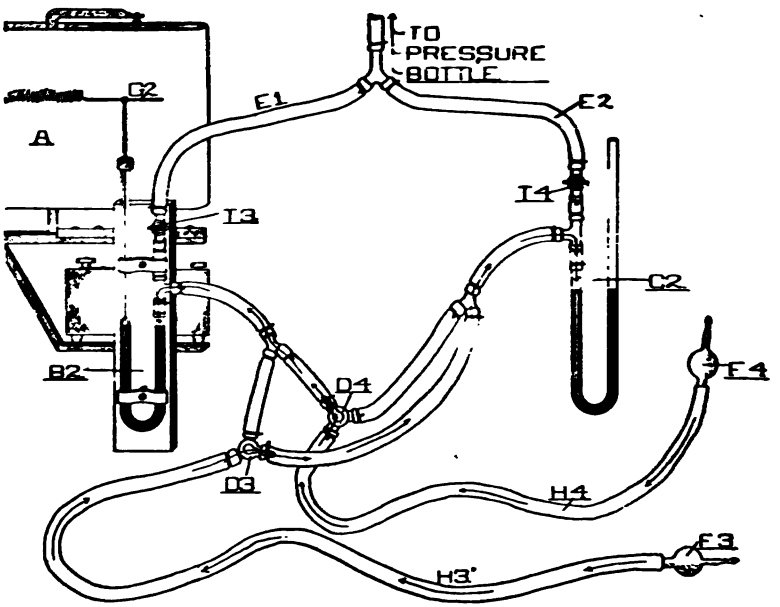

FIG. 30.—A graphic illustration of the arrangement of one-half the apparatus shown in Fig. 29. The letters and figures used in this illustration correspond to those used in Fig. 29.

allow the drum to revolve five, ten and fifteen minutes after the injection. Then by merely reyersing the stopcocks (D_3 and D_4) the dogs can be interchanged, or in other words, the dog which was recording its blood-pressure on the smoked drum will pulsate against the mercury in the "dummy" manometer, and the one which was previously pulsating against the "dummy" will record its normal blood-pressure upon the

smoked drum. After taking a normal tracing several inches in length, stop the drum; then check the former results by injecting this dog with the same preparation given to dog No. 1; again, take tracing five, ten and fifteen minutes after the injection. Repeat operation by injecting, in a similar manner, the other sample into the dogs on the left-hand side of the drum. This will consume about one hour and fifteen minutes. It is then necessary to wait only about fifteen minutes or until the one and a half hours have elapsed since the first injection was given when the entire procedure can be repeated. This is continued until each dog has received three or four injections. The charts are then measured and the average rise in pressure produced by each preparation is taken as its figure of potency.

To assay one sample of ergot in duplicate and one sample of adrenal extract it is necessary to employ only three animals, two on the one end for the ergot and one on the other end for the adrenal extract.

2. **The Cock's-comb Method.**—This method consists in determining the minimum amount of solution of ergot necessary to cause the same degree of bluing in the cock's-comb and wattles as that produced by a given amount of a standard preparation, when introduced into the crop or intramuscularly injected.

This method was formerly regarded as very unreliable because of the difference in susceptibility of various cocks. Some years ago Edmunds was unfavorably impressed with this method and discarded it, but after more extensive experience he stated in a paper with Hale,[1] that accurate results may be obtained by this method if only white Leghorn cocks are used, since the common barnyard fowl varies too greatly in its reaction.

Apparatus Necessary for Experiment.—Accurately graduated syringe for making intramuscular injections and scales with weights up to 2,500 gm.

Animals.—Normal, twelve to eighteen months old, thoroughbred white Leghorn roosters of as nearly the same size and age as possible. Those weighing from 1200 to 1600 gm. are best. The fowls should be allowed to fast for twenty-four hours previous to making the test.

Method of Injecting.—The injections are made deep into the breast muscle, or the preparation to be tested may be introduced into the crop through a soft rubber tube.

[1] Edmunds and Hale, Bull. No. 76, Hyg. Lab. U. S. Pub. Health and Mar. Hosp. Serv.

Actual Standardization.—Inject one cock with a dose of the standard preparation. Inject another with the same dose of the unknown preparation, and, one hour after the injections have been given, compare the color of the two combs, as the maximum cyanosis seems to be reached in about that time. The color begins to fade soon after, so that at the end of two hours or more, depending on the amount of drug given, the comb will usually appear to be nearly normal. Judging from the results obtained from the first two injections, the dose *of the unknown* preparation is increased or diminished until that amount is found which will give approximately the same intensity of action as was shown by the color of the comb of the rooster injected with the standard. The same roosters may be used for several tests, but the injections should be made at intervals of two or three days, so as to allow sufficient time for complete recovery from the effects of previous administrations of the drug. It should not be considered sufficiently accurate if the first injections of the standard and of the unknown preparations produce the same degree of activity, because it is believed that individual variation in different birds might be sufficiently great to interfere. This variation can be reduced to a minimum, if the order of injection on the succeeding day be reversed until each preparation has been given to each of several birds in turn. The results are then based on the average of the several injections. The final comparisons demand careful selection of the birds to be injected. A choice of two birds of about the same size and which had previously reacted well to the drug is advisable. With a little practice it is easy to compare the intensity of the discoloration in the two cocks' combs and to so regulate the dose of the drug given as to produce approximately the same degree of reaction. The doses thus obtained will give the relative strength of the two drugs.

Variations may further be avoided by using only such roosters as react alike to a standard preparation, and also by constantly changing the order of injections so that the same cock shall not receive the same specimen of the drug twice in succession. Although the relative strength of two preparations may be thus determined quite accurately by this method, it is not well adapted for standardization work because it requires that a standard preparation shall be kept on hand. This makes the standard dependent upon the keeping qualities of a stock galenical. Any deterioration, therefore, will result in a lowering of the standard for all subsequent preparations. Owing to the rapid

deterioration in preparations of ergot,[1] it is, therefore, practically impossible to keep on hand a preparation of standard strength and it is believed that more satisfactory results may be obtained by the blood-pressure method because the blood-pressure may be accurately measured by the manometer and kymograph and the same permanently recorded. The principal objection to this method, however, is the fact that the personal equation plays an important part in the assay, since the accuracy of the test depends largely upon the experience of the operator and his ability in determining just when the coloration produced

FIG. 31.—Represents the heads of two roosters. *A* is normal and is given for the sake of comparison. *B* shows the gangrene of the comb and wattles, after an injection of active ergot.

by the unknown equals that produced by the standard. In the hands of an experienced operator, however, results may be obtained which will show, with fair accuracy, the relative value of any preparation of ergot.

3. **Uterine Method** (*in situ*). *Apparatus Necessary for Experiment.*—Myocardiograph, counterbalance weights, surgical instruments, cannulæ, tank for saline bath, all-glass syringe, and an apparatus arranged for maintaining artificial respiration.

Animals.—Various animals may be employed, including the dog, cat, and rabbit, but cats of medium size give the best results.

[1] Recent investigation by Pittenger and Vanderkleed,[2] indicate that ergot fluid extract may be kept *in vacuo* without deterioration. This fact makes it possible to preserve standards with which to compare new lots of the drug and their preparations.

[2] A New and Reliable Method for the Preservation of Ergot Preparations, by P. S. Pittenger and C. E. Vanderkleed, Jour. A. Ph. A., August, 1912.

Preparation of Experiment.[1]—When the intact animal is to be used it is anesthetized with chloretone (0.3 to 0.4 gm. per kilogram of body-weight) given in solution by means of a stomach-tube. A cannula is then placed in the external jugular vein for the injection of the drugs, and a tracheal cannula is inserted to allow artificial respiration, which is kept up during the entire experiment. The animal is then submerged in a 0.9 per cent. saline bath maintained at the constant temperature of 39° C. The further operative process of exposing the uterus is then carried out by an incision along the linea alba from the ensiform cartilage to the symphysis pubes and the two halves of the abdominal wall drawn apart and secured by means of hooks. The bladder and intestines are drawn aside and secured underneath the salt solution to prevent any irritation by exposure to the air and drying. One horn of the exposed uterus is then freed as much as possible from its attachment to the posterior wall of the body-cavity by tearing away the peritoneal attachments. Especially is it advantageous in securing a uterus with much more freedom of movements to detach the ovary by tearing it free from the ligaments binding it to the posterior wall, although care must be used not to injure the blood-supply in these manipulations.

For the purpose of attaching the uterus to the recording apparatus two silk threads, using a fine round needle, are passed through the uterine horn at a distance of about 2 cm. apart. These are attached to the levers of an ordinary myocardiograph.

For recording the movements a light lever is attached to the myocardiograph, and, as the matter of tension is of great importance, a Harvard light muscle lever is made use of on account of its extreme lightness. Since it has been noted that some uteri will react under considerably more tension than others, the amount of tension is varied by the use of counterbalancing weights until a satisfactory result is secured. These conditions are only attained as a result of experiment with each individual organ, and it is frequently necessary to make a number of injections of ergot, using different degrees of tension on the uterus before the best conditions for comparative tests are obtained.

Preparations of Solutions.—All preparations to be tested should be freed from the greater part of alcohol by evaporation on a water bath and made up to fluid extract strength with normal saline solution.

[1] Edmunds and Hale, Bull. No. 76, Hyg. Lab. U. S. Pub. Health and Mar. Hosp. Serv., pp. 28–29.

Method of Injecting.—Same as under Epinephrin Standardization. (See page 52.)

Actual Standardization.—"When the mechanical requirements for the experiments are fulfilled the specimens of ergot are injected. More satisfactory results are secured by comparing only two preparations, the unknown and the standard, at a time, injecting them alternately at intervals of not less than five or, perhaps better, ten minutes, and increasing or diminishing the doses until such amounts of the preparation are found which will produce contractions of equal intensity. It is by no means easy to accomplish this, and frequently it calls for the exercise of considerable patience, as, for example, it is not an unusual experience to carry on such an experiment for two or three hours and, on account of the irritability of the uterus as shown by spontaneous contractions, to know very little more about the relative strengths of the two specimens at the end than at the beginning of the experiment.

"In a favorable experiment, for example, one being carried out on the uterus of a virgin cat, there are no spontaneous contractions, and after each injection the uterus responds by a single contraction. In such an experiment it is often possible to get very definite results in a short time, the results from one pair of injections being verified by further injections until there can be no doubt in the mind of the operator as to the relative activity of the two preparations. No standard of comparison can be given, however, which will fit all animals—in the one case the relative strengths of the contraction as outlined above may be taken, while in the second it may be necessary to adopt as an end-reaction the smallest amount of each drug which will produce a contraction, while in a third uterus, which may be irritable and show spontaneous movements, it may be necessary to employ as a standard the smallest amount which will clearly influence these movements, as, for example, by a delay in the relaxation. Thus it will be seen that each animal appears to be a law unto itself, so far as tension is concerned, and also as to the character of the uterine movements, and that the standard which is to be used in comparing specimens of ergot must be a variable one on that account."

Uterine Method (Isolated).—The uterine methods are based upon the fact that certain drugs have the power of stimulating and thereby increasing the automatic (spontaneous) rhythmic contractions of non-striated muscular tissue. Various methods of standardizing ergot have been devised and employed by various workers utilizing these facts.

In most cases, however, the muscle was suspended in oxygenated Ringer's solution, contained in a Harvard muscle warmer with a capacity of about 40 c.c. and in practically all cases the Harvard light muscle lever was employed for recording the contractions.

The non-concordant results obtained by these methods have been ascribed to the interference of spontaneous contractions and the increasing irritability of the muscle tissue under the continued influence of the drug. These two factors formed the principal objections to uterine

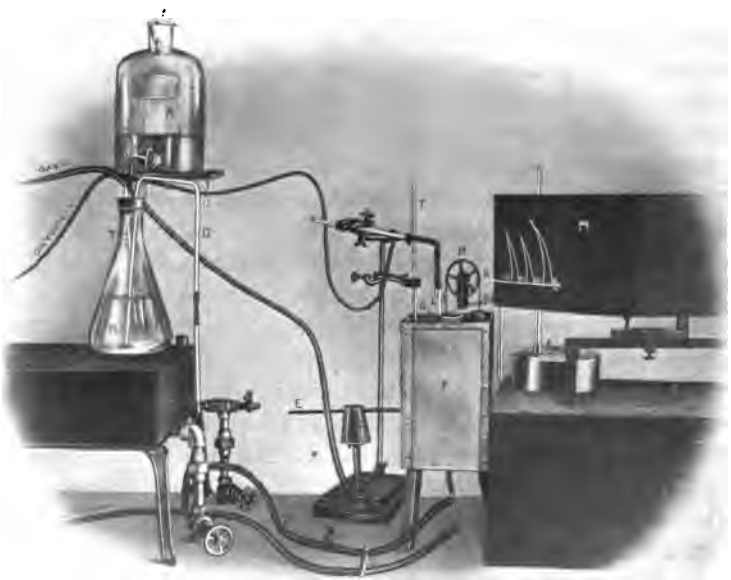

FIG. 32.—Arrangement of the apparatus for recording the contractions of an isolated uterus.

methods. Pharmacologists, therefore, endeavored to overcome these objections by selecting the uteri of animals manifesting the *least degree of normal movements*, preferably those of the cat. But the cat's uterus also proved unsatisfactory. To illustrate, Edmunds and Hale in reporting their observations upon the non-pregnant uteri of cats, state in Bulletin 76, Hygienic Lab. U. S. Pub. Health and Marine Hospital Service: "It is true that the uteri of young cats which may be per-

fectly quiet in the earlier stage of an experiment after some time may begin to contract spontaneously and increase the difficulty of making comparisons of the effects from successive injections of the drug." In such cases the author states, "It may be necessary to employ as a standard the smallest amount which will clearly influence these movements, as, for example, by delay in the relaxation." (See page 73).

Later investigations[1] show that the uteri best adapted to standardization purposes, on the contrary, are those which manifest a ***high*** degree of normal spontaneous movements, preferably those of a non-pregnant guinea-pig, weighing between 275 and 325 gm. Instead of employing a Harvard light muscle lever the free end of the uterus is attached by means of a silk thread to one side of an escapement wheel, to the other side of which is suspended a counterpoise bucket for holding shot. By adding the proper amount of shot to this bucket the operator is enabled to weight the uterus down and thus reduce the amplitude of these movements so they can be controlled. Thus the marked spontaneous contractions can be reduced until the uterus is just able to contract under the increased load, or in other words, shot is added until the maximum amount of work that the uterus is normally capable of performing is counterbalanced. Any increase in the amplitude of the contraction after the addition of a given drug can now be produced only by that drug.

The uterus is suspended in about 250 c.c. of Ringer's solution contained in a cylindrical glass vessel (*G*) the lower end of which is plugged with a rubber stopper (*O*) having a central bore. Through the latter passes one arm of a wide glass "T" tube (*J*) which ends flush with the upper surface of the stopper, so that the cylindrical vessel may be completely emptied. This tube passes through a second rubber stopper (*L*) which fills an opening in the bottom of an outer metallic vessel (*F*) which forms a constant temperature water jacket. The temperature of the water in this jacket is kept constant by means of a metallic rod (*E*) which penetrates the wall of the jacket and passes through the water and is soldered to the opposite side of the jacket. The portion of the rod external to the jacket is heated by a protected Bunsen burner

[1] "A New Uterus-contracting Method of Testing Ergot, with comparison with the blood-pressure method," by Paul S. Pittenger and Chas. E. Vanderkleed. Read at the Sixty-first Annual Convention of the Amer. Pharm. Assoc. held at Nashville, Tenn., Aug. 18–25, 1913.

"The Application of some Muscular Tissues Adapted to Physiologic Standardization," by F. E. Stewart and P. S. Pittenger, Monthly Cyclopedia and Medical Bulletin, Sept., 1913.

(*C*) which slides on the rod. The temperature is regulated by sliding this burner backward and forward until that point is found where the amount of heat transmitted by the rod to the water inside is sufficient to keep the thermometer (*T*) suspended in the water at the proper degree (38° to 39° C.). One of the other arms of the "T" tube is connected by

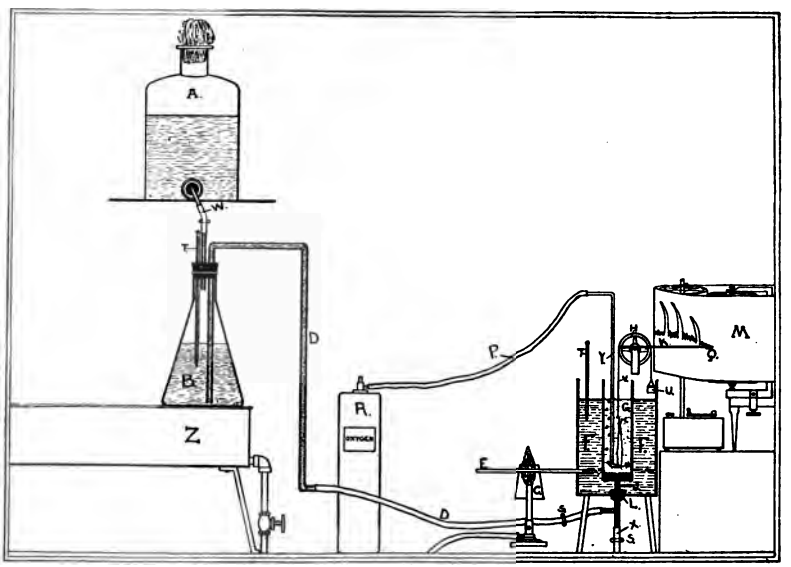

FIG. 33.—A graphic illustration of the apparatus shown in Fig. 32 and will serve to illustrate the following detailed outline of the method. Note that a flat surface is secured at the recording end of the long paper kymograph by the use of an extra single drum kymograph. This is essential in order to record the long, sweeping curves produced by the contraction of the guinea-pig's uterus.

a rubber junction (*X*) armed with spring clamps (*S*) to a waste pipe, by which the cylindrical glass vessel may be emptied. The remaining arm is connected by a siphon tube (*D*) to a flask (*B*) which holds a small amount of Ringer's solution for refilling the cylindrical vessel. This flask is kept at a temperature between 40° C. and 45° C. by means of a steam bath (*Z*).

The main supply of Ringer's solution is contained in a large aspirator bottle (*A*) connected with the small flask by a rubber tube (*W*), the object being to avoid exposing the reserve solution to prolonged heat. Heat causes Ringer's solution gradually to decompose and lose CO_2.

The Ringer solution in the small flask should be reduced to 39° C. immediately before admitting it to the cylindrical vessel by allowing sufficient cold solution to run into it from the aspirator bottle.

Into the cylindrical vessel containing the Ringer solution dips a narrow glass tube (*Y*). This tube is turned at a right angle about half an inch from its lower end. Into this end is sealed a platinum pin (*N*) for attaching the *lower end of the isolated uterus*. The upper end of the tube

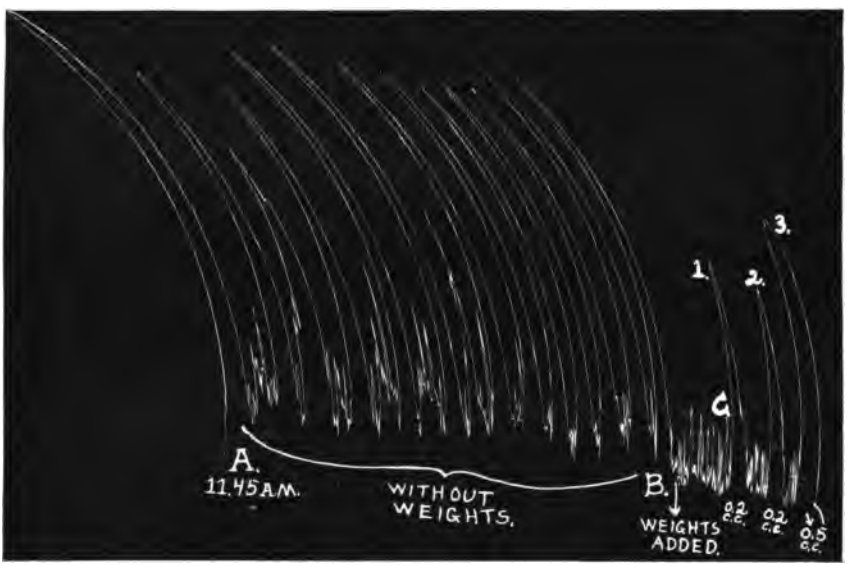

FIG. 34.—Demonstrates first, the normally acting uterus (*A* to *B*); second, the action when weighted down by shot (*B* to *C*); and third, the action of ergot on the isolated uterus when loaded and working against resistance (1, 2 and 3).

is connected by means of rubber tubing, (*P*) to an oxygen reservoir (*R*). A constant stream of oxygen is allowed to bubble through a small vent situated at the lower bend of the tube, thus preserving the muscular irritability of the uterus and at the same time stirring the Ringer solution.

The *other end of the uterus* is fastened to a small platinum hook (*I*) connected to a silk thread (*V*) which passes over an escapement wheel (*H*) and is attached to a pin on the opposite side of the wheel. A counterpoise bucket for holding shot (*U*) is attached to the other side of the

wheel. To this wheel is soldered a stylet of aluminum (*K*), the axle of the wheel serving as a fulcrum. To the end of this stylet a pen point is fixed (*Q*) for recording the contractions of the uterus on the revolving tdrum of the kymograph.

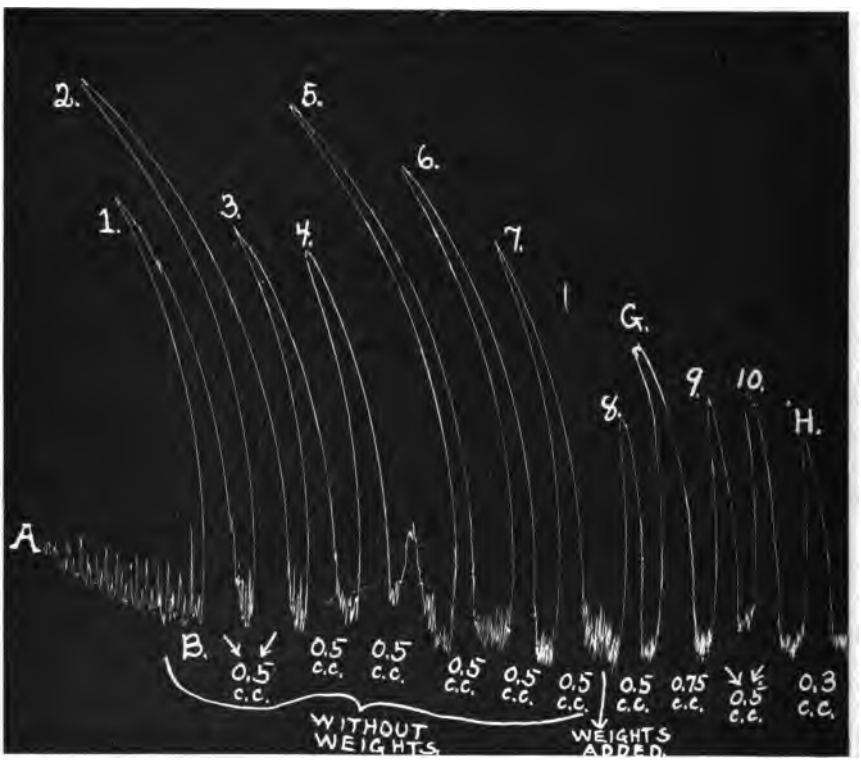

FIG. 35.—Demonstrates first, the normally acting uterus (*A* to *B*); second, the *non-concordant* results produced by repeated doses of the same amount of fluid extract ergot (1, 2, 3, 4, 5, 6, and 7); third, the *concordant results obtained after the uterine contractions are controlled by weights* (8, 9, and 10). The curve *G* and *H* indicate the quantitative results obtained by a larger and a smaller dose.

Method of Procedure.—The animal is bled by quickly severing the carotid artery with a sharp-pointed scissors. The spinal column is then severed with strong scissors. One horn of the uterus is then quickly excised together with the ovary which is left attached by means of the fold of broad ligament in which the Fallopian tube runs. This

horn is then quickly transferred to the oxygenated Ringer's solution in the cylindrical vessel and attached to the two platinum pins above

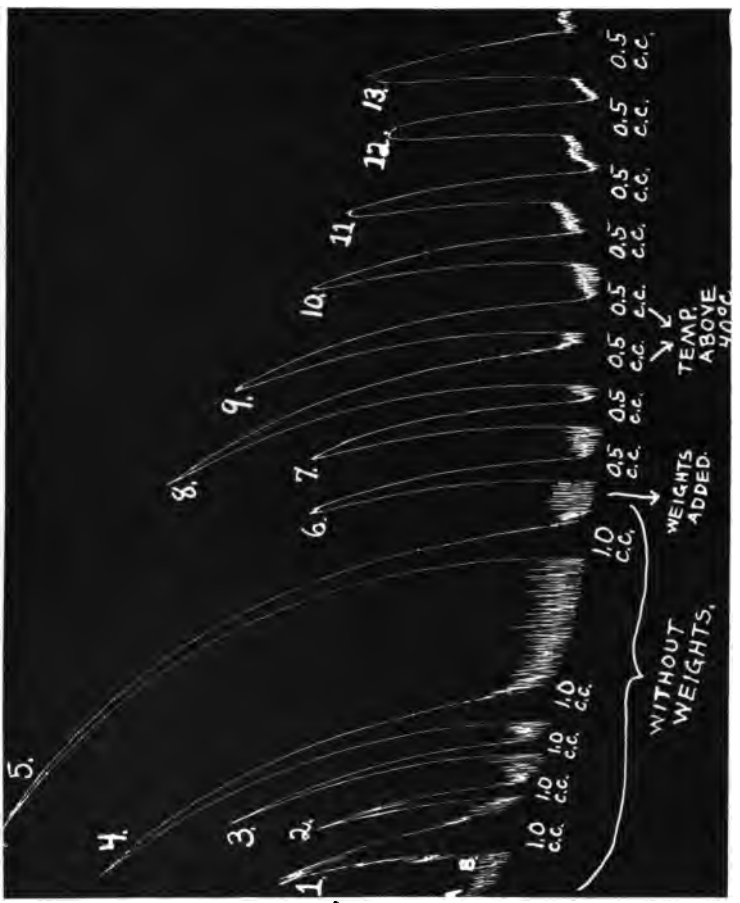

FIG. 36.—This illustration is similar to Fig. No. 35. *A* to *B* show normal contractions; 1, 2, 3, 4, 5, non-concordant results obstained without weights; 6, 7, 10, 11, 12, 13, concordant results obtained after weights were added. The curves 8 and 9 indicate contractions produced under the increased stimulation due to rise in the temperature of the Ringer solution, and show the necessity of maintaining an even temperature during the experiment.

referred to (the ovary is fastened to the hook suspended from the escapement wheel and the lower end of the horn is fastened to the pin at the

lower end of the oxygen tube). The manipulation and exposure followed by the immersion in the warm solution will almost invariably produce a high degree of tonus, which, however, gradually diminishes until the uterus returns to its normal condition. If at this point the uterus does not exhibit *strong* rhythmic contractions it should be discarded and replaced by a new one. The weights are now added by

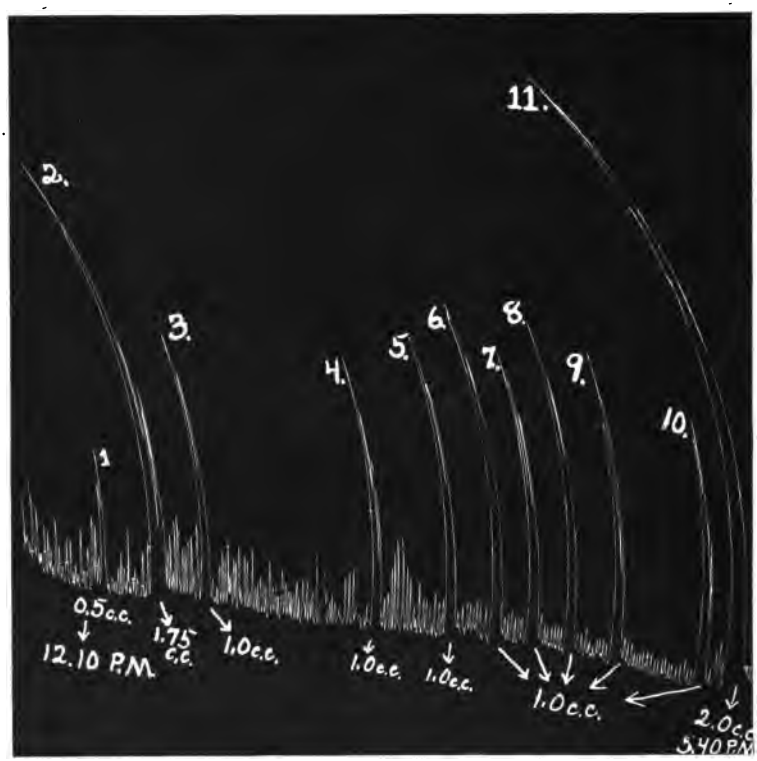

FIG. 37.—This chart clearly demonstrates the concordant results from repeating doses of equal amount. Fig. 1, 2, and 11 indicate quantitative results obtained by varying the doses, and demonstrate the accuracy of this method of standardization.

dropping shot into the bucket *until the uterus can make only small rhythmic contractions.*

Conditions are now suitable for determining the activity of the drug to be tested or standardized. The samples to be tested are first freed

from alcohol by evaporation on a water bath and then made up to their original volume with water.

A small dose (0.3–0.5 c.c.) of the standard preparation is now pipetted into the Ringer's solution in which the uterus is suspended. If all conditions are ideal the uterus which was recording small rhythmic contractions will now forcibly contract and record its contraction by a long sweeping curve. After the curve reaches its maximum and commences to decline (which may require from five to fifteen minutes) the medicated Ringer's solution is quickly run off and replaced by fresh solution, previously adjusted to the proper temperature. The momentary exposure to the air while changing the solution generally causes the uterus to contract rather forcibly, thus markedly increasing the amplitude of the curve produced by the action of the drug. It is necessary, therefore, in changing solutions to hold the escapement wheel for a few seconds or until the uterus is again covered with the saline solution. This will prevent the record from being interrupted by contractions not produced by the drug. The curve now quickly returns to normal and the uterus continues to record its small rhythmic contractions.

Should the uterus chance to be *very sensitive* a dose of 0.5 c.c. of the standard preparation may produce a contraction so strong that it will carry the writing pen off the smoked chart. In such cases it is necessary to reduce the dose. If, however, the uterus still continues to give such marked contractions shot should be added until the contraction can be controlled. On the other hand, should a dose of 0.5 c.c. not produce contraction, the dose should be increased to 1 or 1.5 c.c. If, however, doses of 1.5 c.c. do not call out contractions, shot should be removed until a marked contraction is produced by these doses.

After thus adjusting the apparatus two successive doses of equal amounts of the standard solution should be administered. If the resultant contractions are equal the uterus is giving concordant results and is ready for assay purposes. In order to determine the relative activity of an unknown preparation it is now merely necessary to give progressively increasing or decreasing doses of the unknown preparation until that amount is found which will produce contractions of an equal amplitude as those produced by the standard preparation.

Description of Charts.—The terms "no weights" and "weight added" used in the descriptions of the charts refer to the shot used in inhibiting the normal contraction of the uterus, not to the counterpoise employed to keep the uterus suspended in the Ringer's solution.

Uteri differ greatly in their mutual relation as to power, and muscular structure. (See Fig. 38.) Some specimens are greatly deficient in muscular substance and act feebly while other specimens show greater muscular development and contract strongly. Some specimens prove absolutely inert and will not respond at all. The normal activity, however, practically runs parallel with the amount of muscular tissue present; the "stringy" uteri are all deficient in normal activity and in response to stimuli, while the thick, more muscular uteri are practically all active and sensitive. This knowledge enables the operator to save considerable amounts of time as it renders it possible for him to distinguish between active and inactive uteri before connecting them with the apparatus.

Due to the marked differences in the sensitiveness of the various uteri, it is necessary to employ a standard preparation with which to compare the unknown preparations. These comparisons must of course always be made on the same uterus.

As a standard we employ a fluid extract of ergot of such strength that when injected intravenously into a series of two or more dogs it produces an average rise in blood-pressure of 30 mm. of mercury. After preparing a standard preparation of the above strength it is placed into 4 c.c. vacuum ampuls to prevent deterioration.[1] These can then be opened and used as required.

The Ringer's solution employed in these experiments should be made according to the formula of Loche (see page 143) and should be kept sterile as the presence of bacterial growth in the solution immediately gives rise to non-concordant and unsatisfactory results.

[1] See page 9.

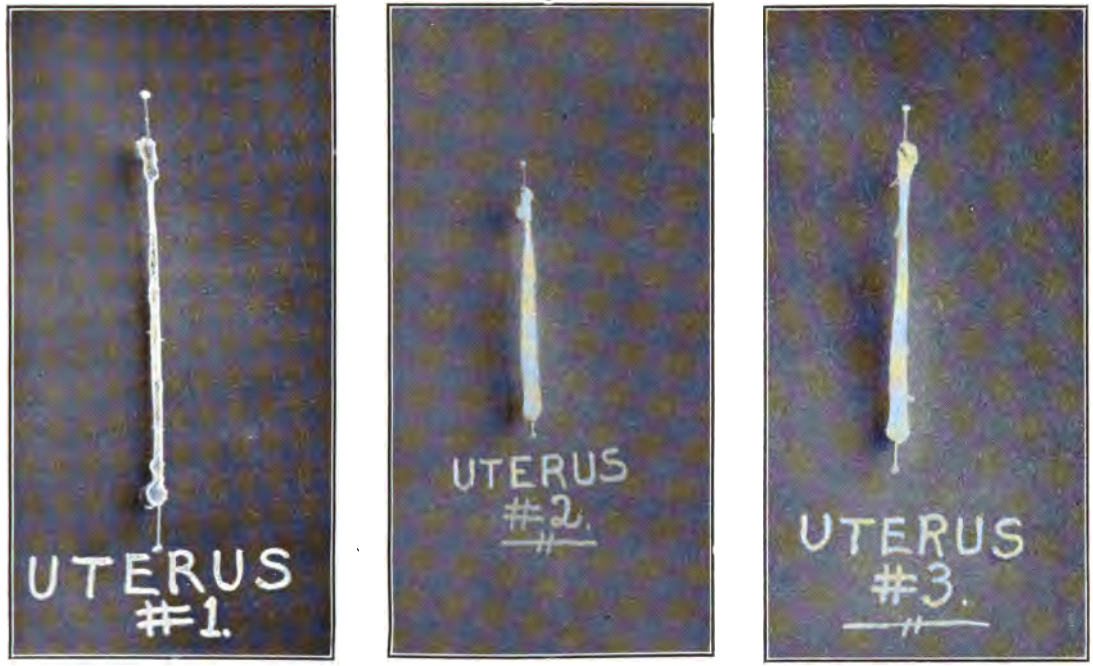

FIG. 38.—Illustrates the variation in muscular structure of different uteri. The above uteri were all taken from guinea-pigs weighing from 280 to 320 gm.

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CHAPTER V

THE PITUITARY BODY

The role which the pituitary body or hypophysis plays in life has until recently been a mystery. It was at first thought that its function was to lubricate the nasal cavities. This belief, however, was soon discarded and replaced by the supposition that the gland was, like the appendix, of no use at all. Later, however, it was proven by Vassale and Sacchi,[1] Caselli,[2] and others that the gland plays a very important role

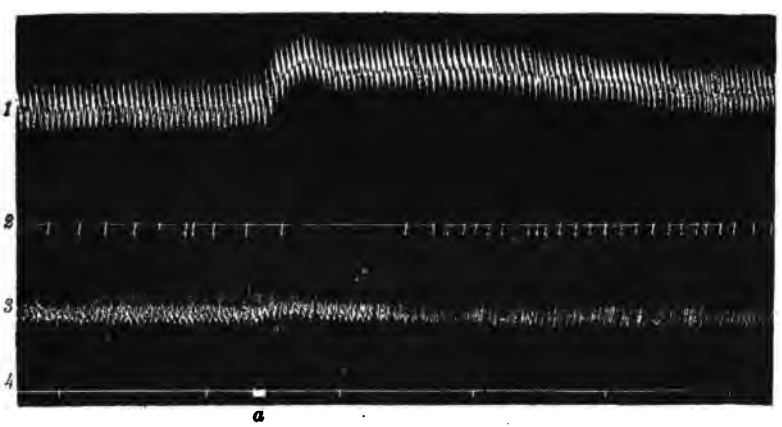

FIG. 39.—Effect of pituitary extract on diuresis. At *a* 0.3 c.c. pituitrin was injected intravenously ("repeat dose"). 1, Carotid blood-pressure; 2, urine registered by drops; 3, carotid pulse; 4, time, one minute. (Hoskins and Means.)

and is **absolutely necessary to life.** It has also been found that acromegaly and other diseases are due to functional disturbances produced by an over or an under secretion of this gland and that its removal causes death. According to Sajous[3] the anterior lobe may prove to be the center of the adrenal system.

[1] Vassale and Sacchi: Rivista Sperimentale de Freniatria, p. 83, 1894.
[2] Caselli: Studii anatomici e sperimentali sulla Fisiopatologia della Glandola pituitaria, 1900.
[3] Sajous: Internal Secretions and the Principles of Medicine, vol. 1, p. 216.

The pituitary body varies in **size** according to the age and species of the animal. The gland most commonly used in therapeutics is that obtained from the ox, and is about 3/4 in. in diameter.

FIG. 40.—Effect of pituitary extract on the isolated uterus.

The gland is **composed of two parts or lobes**—the anterior and the posterior or infundibular. The smaller or posterior lobe, which forms only about 10 to 15 per cent. of the total gland, is the more important

therapeutically. This lobe contains practically all of the active principles while the anterior lobe is the one which is so necessary to life.

The total pituitary body contains about 80 per cent. of water, or in other words 100 parts of the fresh gland give about 20 parts of dry substance, containing 2 to 3 parts of the posterior lobe.

Knowledge concerning the chemical composition of the pituitary gland has only recently gained proportions sufficient to warrant the hope that science will ultimately be as successful in isolating and synthesizing its active principle or principles as it has been with the suprarenal gland. Owing to the similarity existing between the physiologic actions of the pituitary and those of the suprarenal gland the theory has been advanced that the active principles of the former will be very similar to epinephrin. In fact it has been shown that the activity of the gland can be concentrated into a basic fraction forming salts with acids. It was possible, however, to split this basic fraction into several fractions of different chemical properties (Fühner),[1] which would tend to

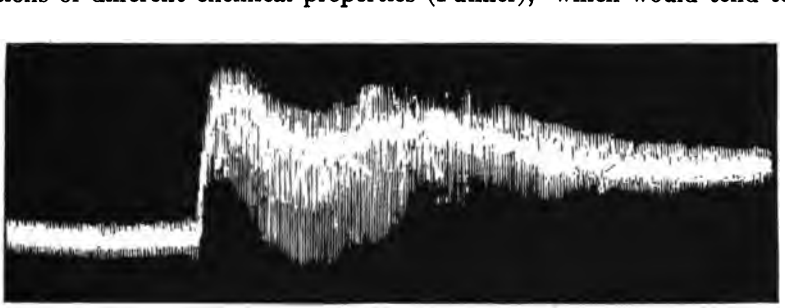

FIG. 41.—Effect of pituitary extract on the blood-pressure.

prove that the action of the pituitary body is due to not one but to the combined actions of several active principles. Schäfer and Vincent[2] had also shown some time before that the blood-pressure-raising principle could be divided into two fractions by their different solubilities in alcohol, one containing a pressor and the other a depressor action.

Physiologic experiments have demonstrated that extracts of this gland are valuable therapeutic agents. Thus Magnus and Schäfer[3] and Schäfer and Herring[4] have shown that it **accelerates diuresis**; Oliver and Schäfer[1] that it is valuable for **raising the blood-pressure by**

[1] Fühner: Deutsche medizinische Wochenschrift, March, 1913, p. 491.
[2] Schäfer and Vincent: Journal of Physiology, May 11, 1899.
[3] Magnus and Schäfer: Proc. Phys. Soc., p. 11, 1901.
[4] Schäfer and Herring: Phil. Trans., 1906 B.

arterial constriction (see Fig. 41); Dale,[2] Bell and Hick,[3] and v. Fränkl Hochwart and Fröhlich[4] that it **excites marked uterine contractions** (see Fig. 40), Ott and Scott[5] that it possesses a rather **marked galactagogue action.**

STANDARDIZATION OF PITUITARY EXTRACTS

The scant knowledge of the chemical composition of the gland and extracts of the same renders it impossible to ascertain by chemical means the comparative value of two or more extracts or fractions. We are therefore compelled to resort to physiologic assay methods. Of the various physiologic actions of the gland above mentioned there are three, which present themselves as possible means of physiologic standardization, *i.e.*, the action on the blood-pressure, the uterus, and the kidneys.

Blood-pressure Method for Standardizing Pituitary Extracts

Apparatus Necessary for Experiment, Animals, Preparation of Experiment, Method of Injecting.—Same as under "Epinephrine Standardization," see page 52.

Actual Standardization.—After all preliminary arrangements have been completed about 0.05 c.c. of the standard extract should be injected into the femoral vein. If this produces a rise in blood-pressure less than 24 mm. (12 mm. as recorded by the U-shaped manometer) the dose of the standard extract should be increased. If on the other hand the injection of 0.05 c.c. produces a rise of more than 40 mm. the dose should be decreased, as large doses markedly influence the succeeding injections. (See Fig. 42.) After the "standard dose" of the standard solution is thus determined a "standard dose" of the unknown solution is injected and the rise in pressure compared with that produced by the standard solution. If the difference is very great the unknown solution is strengthened or diluted as the case may be. The size of the injection is then increased or decreased until that dose of the unknown solution is found which will cause the same rise in pressure as that produced by the "standard dose" of the standard solution. Occasional injections of the standard solution should be made to show the variation in the reaction of the animal, due to the effects of previous injections. Final

[1] Oliver and Schäfer: Journ. of Phys., XVIII, p. 277, 1895.
[2] Dale: Biochem. Journ., IV, p. 427, 1909.
[3] Bell and Hick: Brit. Med. Journ., I., p. 777, 1909.
[4] v. Fränkl Hochwart and Fröhlich: Arch. f. exp. Pathol. u. Therap., LXIII, p. 347, 1910.
[5] Ott and Scott: Proc. Soc. Exp. Biol., New York, 1910.

equality is secured by injecting alternately a standard and the unknown solutions until the average rise of several consecutive injections is practically equal.

The fact that the blood-pressure method involves the simplest technique together with its satisfactory and almost universal use as a means of standardizing epinephrine and suprarenal extracts would at first lead one to believe that this method would also be the most satisfactory one for standardizing pituitary extracts. It has, however, serious disadvantages in the latter case. As before stated the blood-pressure-raising principle can be divided into two parts, one possessing a pressor and the other a depressor action. Prof. Fühner of Frieberg[1] has also

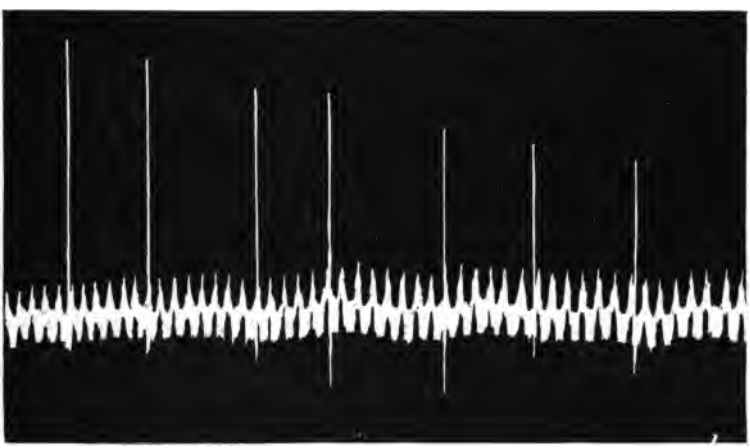

FIG. 42.—Shows the gradual decrease in the rises in blood-pressure produced by repeated injections, of equal size, of pituitary extract.

shown that the sum of the basic principles tested by him caused marked uterine contractions and only a slight pressor action, which was almost completely masked by a marked preliminary depressor action.

Furthermore extracts which have been deprived of their depressor action by fractionation with alcohol showed marked pressor effects, while on the other hand, they were sometimes almost entirely free from action on the uterus.

Another serious drawback to the blood-pressure-raising method is the fact that the active principles of pituitary extract are not nearly as rapidly oxidized as those of the suprarenal gland and therefore repeated

[1] Deutsche medizinische Wochenschrift, 1913, No. 11, p. 491.

injections of equal sizes produce unequal rises, the subsequent ones generally showing a waning of the pressor action and an increasing prominence of the preliminary depressions.

Still another objection to the blood-pressure method is its comparatively low sensitiveness; in other words it requires, in most cases, a rather large variation in the size of the injection to produce a variation in the resultant rise. This latter objection is especially serious when comparing two or more samples for research purposes, in which case a mistake of 20 to 30 per cent. in interpreting the results of an assay may cause a considerable loss of time. The greater sensitiveness of the uterine method is shown by Figs. 43 and 44, which show the results obtained from tests upon both the blood-pressure and isolated uterus methods, in

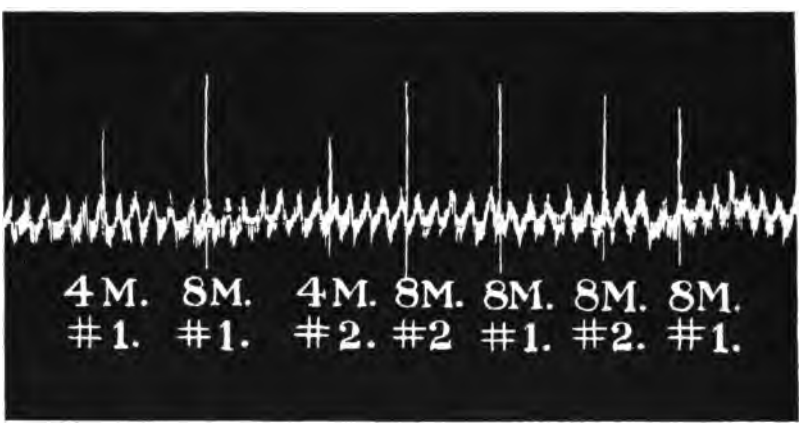

FIG. 43.—Tracing showing equal rises in blood-pressure from equal amounts of the two extracts of pituitary mentioned above.

order to determine which was the better of two pituitary extracts made by different processes. According to the blood-pressure method both extracts showed the same activity while when tested by the uterine method, which is far more sensitive, 8 minims of one extract proved to be more active than 12 minims of the other.

It has been shown by Dale and Laidlaw that **methods of standardization based upon the diuretic action of pituitary extracts** are also unsatisfactory because of the tolerance produced by the first injection. They state that if small doses are used in order to overcome this tolerance, "it may be difficult to distinguish genuine effects from the spon-

taneous variations of urinary flow which occur in almost any experiment however constant the controllable conditions."

Isolated Uterus Method of Standardizing Pituitary Extracts

The action upon the isolated uterus has, in the hands of most workers, proven to be a very satisfactory method of valuating pituitary extracts. By this method a small uterine muscle is suspended in a comparatively large volume of oxygenated Ringer's solution, which can

FIG. 44.—Tracing proving the sensitiveness of the uterine method. Chart shows 8 minims of extract No. 1 to be stronger than 12 minims of extract No. 2. By the blood-pressure method (owing to its lower sensitiveness) both preparations show the same activity. (See Fig. 43.)

be readily removed and replaced by a fresh volume. This gives a much better chance, than presented by other methods, for the tissue to recover its original condition by the rapid washing out of the principle or the reduction of its concentration to below the threshold of activity.

Apparatus Necessary for Experiments, Animals, Method of Injecting, Preparation of Experiment, and Technique.—Same as under "Isolated Uterus Method for Standardizing Ergot." (See pages 73 to 83.)

Preparation of Standard Solution.—Due to the variation in susceptibility of the different uteri (see Fig. 38, page 83), this method must essentially be comparative and not absolute. This necessitates the adoption of an arbitrary standard with which the activity of the unknown can be compared.

This is a point on which some generally acceptable convention is ultimately to be desired, but further experience with the conditions of stability is needed before any definite recommendation can be given. Schäfer and Herring state that the fresh infundibular substance, dried at a low temperature, is indefinitely stable, and it may be that ultimately a freshly prepared decoction of such a dried material will prove to be the best standard of reference. But Schäfer and Herring's statement was evidently based on general impression—the effects produced, that is to say, did not become obviously weaker on the average. The question is by no means a simple one for no absolute reference exists, on the one hand, which can be with certainty reproduced, while, on the other hand, the available methods of evaluation are essentially comparative. The variation in the responsiveness of different individuals, or different isolated uteri, is very wide, so that neither the minimal effective dose nor the magnitude of response to a dose of standard size gives any useful information. For the present we use as a standard the extract prepared by a brief boiling of the perfectly fresh and finely pounded infundibular material with a definite proportion of acidulated water, so as to produce a 10 per cent. or 20 per cent. extract of the fresh moist substance. The extract is then sterilized by brief autoclaving in small phials. Some activity is lost in autoclaving, but the preparation thereafter has great stability. The use of small phials, of which one can be used for each test, obviates repeated sterilization, which is inadmissible. At frequent intervals a new batch is chosen which has an activity equal to or just perceptibly greater than that of the former standard, so that the possibility of a very slow lowering of the standard is eliminated, though we have as yet no evidence of deterioration under such conditions (Dale and Laidlaw).

Actual Standardization.—After the uterus has been placed in the apparatus and has attained the condition of uniform low tonus, a small dose (about 0.01 c.c.) of the standard extract is pipetted into the

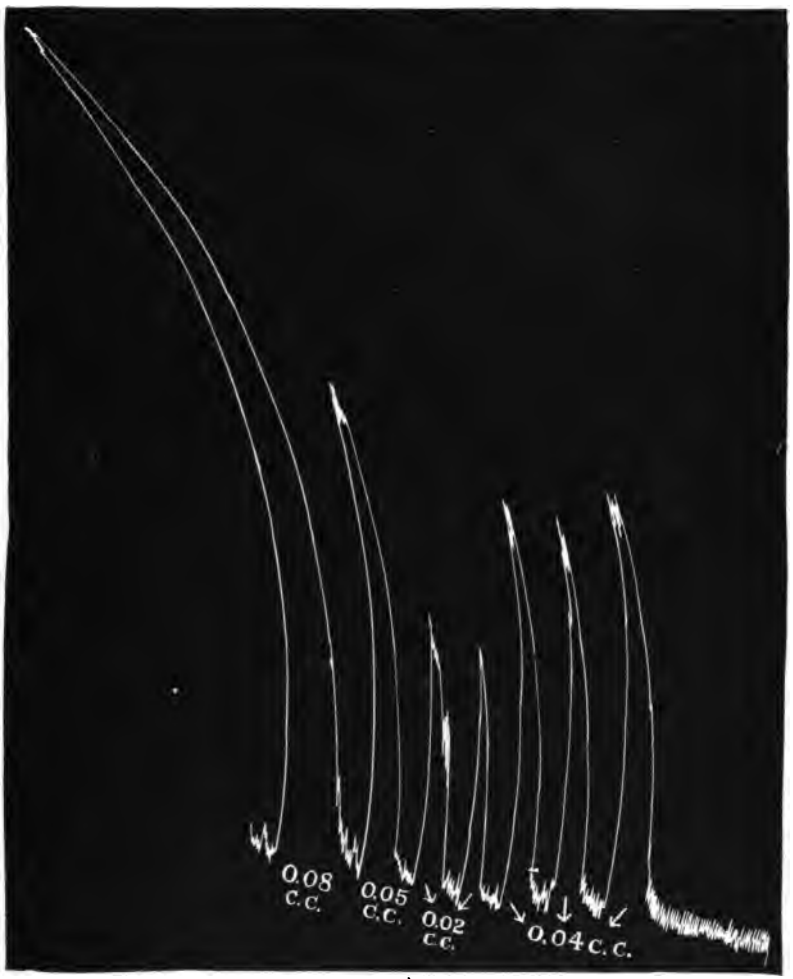

FIG. 45.—Demonstrates the concordant results obtained from repeated doses of an equal amount of pituitary extract.

Ringer's solution in which the uterus is suspended. If all conditions are ideal the uterus which was recording small rhythmic contractions will now forcibly contract and record its contraction by a long sweeping curve. The dose should be sufficiently large to produce a nearly but

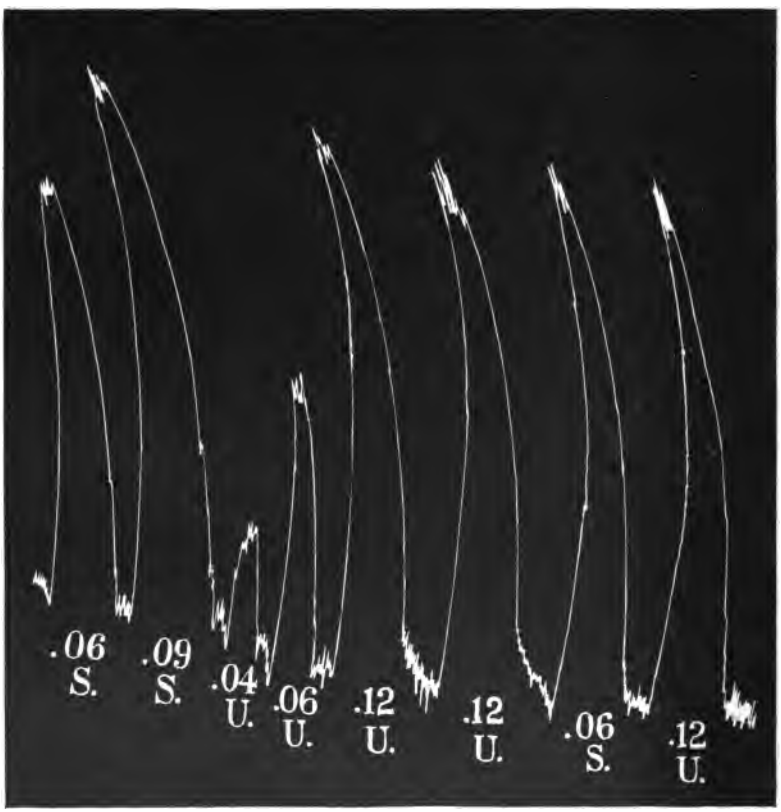

FIG. 46.—Tracing showing the results of an assay of pituitary extract on the isolated guinea-pig's uterus. *S*, standard extract; *U*, unknown extract; tracing shows 0.06 c.c. of the standard extract to be as active as 0.12 c.c. of the unknown extract.

not quite maximal tonus. If this initial dose of the standard extract produces a maximal tonus the drugged solution should be quickly removed and replaced by fresh Ringer's, after which a smaller dose is tried. If on the other hand, the lever after rising to a position of partial tonus falls again and a wide rhythm ensues, the dose is too small. In such cases the solution should be changed and after about ten minutes at

minimal tonus, larger doses should be tried. If, however, doses of 0.03 c.c. do not produce tonic contractions, the uterus should be discarded and replaced by another. After thus determining the "standard dose" of the standard extract, a "standard dose" of the unknown extract should be given and the resultant contraction compared with that produced by the standard extract. If the difference is *very* great the unknown solution is strengthened or diluted accordingly. The size of the injections is then increased or decreased until that dose of the unknown is found which will cause a contraction of an amplitude equal to that produced by the standard. Occasional injections of the standard solution should be made to insure constancy in the reaction of the uterus. Final equality is secured by injecting alternately the standard and the unknown extract until the average amplitude of the curves produced by several consecutive injections is practically equal.

It is not necessarily significant that there occur a slight variation in the amplitude of the curves produced by two successive doses, as a second equal dose of the same preparation will frequently give a contraction of slightly greater amplitude than that produced by the first dose, or may, on the other hand, produce a slightly smaller one. The uniformity of result is more nearly complete with some uteri than others. The uniformity depends to a considerable extent on the *uniformity of interval between the doses*, so that the first dose of a group, either at the beginning of the experiment, or at a later stage following a prolonged interruption, tends to produce a slightly abnormal result in one direction or the other. The succeeding doses, however, generally give concordant results. On the other hand it is inadvisable to give a long series of doses at short intervals as this produces a gradual decline in the sensitiveness of the uterus. It is advisable, therefore, when making a series of comparisons, to give a group of four or five doses at about ten-minute intervals, then allow an interval of about one-half hour or so and then follow with another group, regarding the first member of each group of curves as probably abnormal. Good uteri will give reliable results for eight to ten hours after being excised and placed in the oxygenated Ringer's solution.

This method is by far the best so far proposed, for the standardization of pituitary extracts, as differences of activity which are only just appreciable by the blood-pressure method, under the best conditions, are at once obvious in the test on the uterus without any special care in controlling the regularity of the response.

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CHAPTER VI

CANNABIS SATIVA

There are several commercial varieties of Cannabis sativa on the market, the most important of which are the Indian, African, Australian and American.

Formerly it was the general belief that only the Indian variety of Cannabis was of value therapeutically. It was also believed to be a distinct species, but it really differs so little from the other forms that botanists now consider them as merely varieties.

There exists a difference of opinion as to the comparative physiologic activity of the different varieties. So far as assays on dogs are concerned, there seems to be very little choice between them (see Table VII), but preliminary investigations on horses now under way indicate that there may be some difference with these animals especially when the drug is administered intravenously.

TABLE VII.—THE COMPARATIVE RESULTS OF BOTH CHEMIC AND PHYSIOLOGIC ASSAY OF THE PRINCIPAL THREE VARIETIES OF CANNABIS SATIVA

Cannabis indica		Cannabis africana		Cannabis americana	
Chemical assay, per cent.	Physiological assay, per cent.	Chemical assay, per cent.	Physiological assay, per cent.	Chemical assay, per cent.	Physiological assay, per cent.
12.2 resin.....	.....	10.6 resin...	100	6.4 resin...	133
12.7 resin...	.....	16.77 resin..	133.3	12.46 resin..	100
14.25 resin.....	100	14.2 resin...	133.3	14.09 resin..	100
12.81 resin.....	100	8.6 resin...	100	12.94 resin..	133
13.04 resin.....	80	10.0 resin...	133	5.29 resin..	100
13.4 resin.....	66	17.5 resin...	Less	6.55 resin..	100
14.0 resin.....	160	.....	than 20	10.79 resin..	160
10.38 resin.....	133	18.79 resin..	100	.....	250
15.07 resin.....	133	17.78 resin..	83	.....	114
.....	200	10.71 resin..	133	17.06 resin..	114
.....	140	.....	.....	10.87 resin .	260
13.02 resin.....	133	.....	.....	8.53 resin..	114
.....	200	.....	.....	.....	.....
.....	266	.....	.....	.....	.....
.....	200	.....	.....	.....	.....
9.87 resin.....	125	.....	.....	.....	.....

Table VII shows that although there seems to be little choice between the different varieties of Cannabis, there *is* a marked variation in the activity of different samples of the *same* variety. It also shows a large variation between the chemic and physiologic results, thus proving the fallacy of the common practice of standardizing preparations of Cannabis according to their resin content. It can readily be seen, therefore, that the only reliable index to the therapeutic value of this drug is the *physiologic* assay.

H. C. Wood[1] states, "Extract of hemp is a very unsatisfactory drug from the fact that 1/8 of a grain of one extract will produce decided intoxication, and many grains can be taken of another extract that cannot be distinguished physically or chemically from the first specimen. The only way of using it with advantage is for the practitioner to try various samples in ascending doses, and use those which are active in the dose which he has found to be effective." This objection, however, can be entirely eliminated by physiologic standardization as by this means every preparation can be brought to the same strength *before* its use on the human.

STANDARDIZATION OF CANNABIS SATIVA

This assay depends upon the characteristic action of the drug upon the central nervous system, in which changes are induced causing a peculiar train of symptoms. These are divided into three typical stages: (1) one of excitability; (2) one of inco-ordination; (3) one of lassitude and sleep.

Shortly after receiving a dose of suitable size of cannabis indica the animal generally vomits and then becomes excitable. In from one to two hours inco-ordination follows; the dog loses control of its legs and of the muscles supporting the head, so that, when standing, the feet are usually spread apart to maintain balance. (See Fig. 48.) When nothing occurs to attract attention the head droops and body sways from side to side or anteroposteriorly. A distinct ataxia is present when walking, and, when severely affected, the animal will stagger and fall. If, however, it is spoken to sharply or attention is drawn to anything of interest it may momentarily recover and the typical effects of the drug disappear, but in a few minutes again relapses into the former condition. As the action of the drug progresses the muscular inco-ordina-

[1] Wood: Therapeutics, Its Principles and Practice, p. 117, 14th edition.

tion becomes greater and the animal passes into the third stage; the depression and lassitude are increased until finally the animal sinks to the floor as if exhausted and passes into deep, undisturbed sleep. After

FIG. 47.—Normal dog.

several hours the effects of the drug slowly disappear and the normal condition returns.

FIG. 48.—Same dog as shown in Fig. 47, one hour after receiving a dose of active cannabis indica. This figure clearly illustrates the stage of inco-ordination produced by cannabis. That the dog has lost control of the hind legs and of the muscles supporting the head can be noted by the drooping of the head and hind quarters. Also note that the legs are spread apart in order to maintain balance.

For standardization purposes the end-reaction to be observed is one just sufficient to produce muscular inco-ordination in a dog.

Animals.—Short-haired dogs of medium size (6 to 12 kilos) are well adapted for this work. They show the different stages of the drug's action because of their comparative high cerebral development.

Animals for assay purposes should be selected with great care, it being necessary to pick out those that are healthy, intelligent, quiet, and which have shown by previous tests that they are easily susceptible to the action of the drug.

After several dogs have been selected, the operator, before using them for actual work, should study each animal in order to familiarize himself with the behavior, peculiarities, etc., of the dog under normal conditions. The same animal may be used many times, provided that twenty-four to thirty-six hours are allowed to elapse between doses in

FIG. 49.—Same dog as shown in Figs. 47 and 48, one and a half hours after receiving a dose of active cannabis indica. This figure shows the animal when severely affected, and about to fall foward.

order that the animal may *completely* recover from the effects of the previous dose.

Although the animals never appear to lose their susceptibility, it is not advisable to use a dog for more than six months, and care should be taken to allow one week to elapse between assays.

Preparation of Experiment.—Select and weigh several animals which have been found easily susceptible to the action of cannabis, and withhold all food for at least twelve hours previous to the time of administration of the drug. Water should be allowed.

Preparation of Drug for Administration.—Tinctures, solid, powdered,

and fluid extracts, are weighed or measured directly into hard gelatin capsules. When a crude drug is to be tested a representative sample should be finely ground and then made into a fluid extract.

Method of Administering.—The drug is administered internally by means of a small capsule. The animal's mouth is opened by forcing the thumb and index finger of the left hand between the jaws, back of the

FIG. 50.—Method of administering capsules to dogs.

teeth. The capsule is then placed on the back of the tongue with the right hand and the mouth quickly closed; while still holding the mouth shut, the animal can be made to swallow the capsule immediately by slapping it on the throat.

Actual Standardization.—Administer to a series of three selected dogs 9/10, 10/10, and 11/10 of the standard dose of the preparation to be tested, for each kilo body-weight of animal. The animals are then placed in a room where they will be undisturbed and are remote from noise and excitement; careful observations should be made and the results recorded during four or five hours.

If this preliminary test shows that the drug is either above or below standard strength other dogs are given progressively increasing or decreasing doses, as the case may be, until the smallest dose per kilo body-weight is found which will produce an action just sufficiently pronounced to bring on the stage of inco-ordination. This is distinguished by a slight ataxia when walking and a drooping of the head and gentle swaying of the body while at rest. The relative strength of the preparation tested is then computed between the "minimum dose" and the "standard minimum dose" by simple proportion.

The personal equation plays an important part in this assay, since the accuracy of the test depends largely upon the experience of the operator and his ability in determining just when the effects of the drug manifest themselves. In the hands of an experienced operator, therefore, results may be obtained which will show, with fair accuracy, the relative value of any preparation of cannabis sativa.

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CHAPTER VII

TECHNIQUE AND APPARATUS EMPLOYED

The purpose of this chapter is to give a description of the technique and apparatus employed in carrying out biologic assays. In order to familarize the student with all the more important apparatus used in experimental physiology, descriptions are also included of apparatus employed in demonstrating the *qualitative* physiologic action of drugs.

A large percentage of the apparatus used is that designed by Dr. William T. Porter and manufactured by the Harvard Apparatus Company. As I could not be expected to improve upon the descriptions of the designer I have in many cases quoted the descriptions of the apparatus devised by Dr. Porter from his "*Introduction to Physiology.*"

FIG. 51. All-glass syringe.

All-glass Syringe.—A syringe composed of three parts—*A*, needle head; *B*, plunger; *C*, graduated barrel. The sides of *A* and *B* are ground to fit eaccurately within the ground inner surface of *C*; the inner ends of *A* and *B* are also ground to fit into each other, thus ensuring complete emptying of the syringe and eliminating all possibility of leakage around the plunger. This syringe is particularly adapted for injecting small doses. It is especially valuable in intravenous work as it permits the operator to observe when all the air has been expelled from the syringe—a factor of the utmost importance in this class of work.

Anesthetic Bottle and Air Warmer.—*For use in connection with the Respiration Pump.*

Referring to the above illustration, it will be seen that the bottle is similar to an ordinary specimen bottle with ground top, which is held up against a flat plate by a moveable bridge piece and screw, it being only necessary to loosen the screw about two turns when the bottle can be removed for filling or cleaning. On the top of the plate is the

regulating cock, which regulates the amount of air (and consequently the strength of the anæsthetic) which passes through the bottle. Should the anæsthetic become used up during an experiment, it is only necessary to push the handle over till the pointer is at *O* of the graduated

FIG. 52.—Palmer's anesthetic bottle and air warmer.

scale, when the bottle is entirely shut off, and can be removed for refilling: all the air passing direct to the heater, which consists of a brass tube with moveable ends, large enough to hold two ordinary electric lamps, there being two holders of the standard bayonet type provided, also two switches. It is advisable to have the lamps of different powers, say an 8 and a 16 C.P., then either the 8, the 16, or both, can be used according to the amount of air and degree of heat required. A little thick grease (as is the common practice in air pump experiments) may be smeared on the plate to make the joint round rim of bottle perfectly air tight. Depending from the plate into the bottle is a tube which

causes the air to "blow" on to the surface of the anæsthetic, and as the latter becomes used up means are provided for lengthening this tube from outside the bottle.

Animal Holders.—In all experiments requiring operations upon anesthetized animals, the animals should, for convenience, be tied to a board.

(A) *Cats.*—It is best first to place cats in a metal etherizing box until under the influence of the anesthetic. They are then placed on a

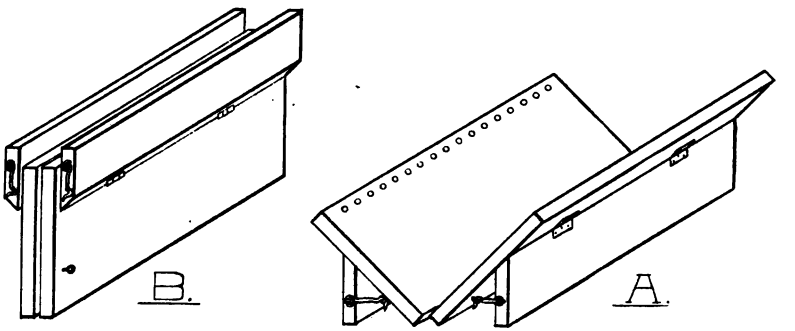

FIG. 53.—Simple, efficient and inexpensive folding animal holder, easily made from four pieces of board, six hinges and two hooks and eyes. *A*, open; *B*, folded.

Harvard animal board, the head of the animal being held in position by means of a Czermak headholder.

(B) *Dogs.*—There are many complicated and expensive dog boards upon the market, but a simple V-shaped trough, with a series of holes along the upper edges through which the cords binding the dog are

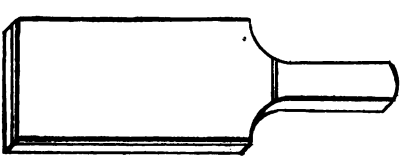

FIG. 54.—Frog board.

passed, answers all ordinary requirements. This series of holes extends the full length of the board thus making it possible to conveniently fasten any size dog to the board.

(C) *Frogs.*—The most convenient holder for frogs is a cork-covered board shaped as shown in the accompanying illustration. These boards should be about 5 in. × 7 in. with a 4-in. handle.

(D) *Rabbits.*—A small dog holder as shown above answers all ordinary requirements.

Artificial Respiration.—In intact animals pull the tongue forward to prevent any hindrance to the entrance of the air into the windpipe,

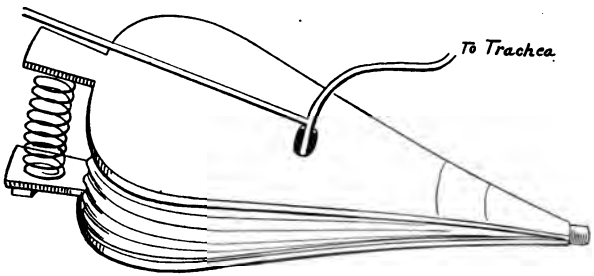

FIG. 55.—Bellows for artificial respiration. (Sollmann.)

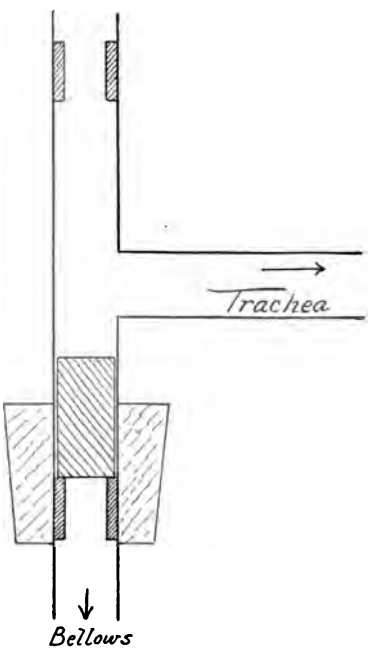

FIG. 56.—Hall's respiration valve. Natural size. (Sollmann.)

then apply with the hands gentle intermittent pressure on the chest and abdomen. Care should be taken not to apply too much pressure as this is liable to rupture the lungs.

Artificial respiration is maintained during operations upon animals by means of some mechanical apparatus connected to the trachea by means of a cannula. "The simplest device consists in a large bellows (15 by 22 in., exclusive of the handles). This may be arranged for foot power by fastening a spiral upholsterer's 'lounge spring No. 2' between the handles. The spout is closed with a cork. An inch hole is bored in the top. This bears a perforated cork, from which a tube leads to the

FIG. 57.—Dr. Broidie's respiration pump.

tracheal cannula. A 'T' piece is inserted in the course of this tube, the free limb of the T being closed when the air is driven into the lungs, and opened when it is expelled. This may be done with the finger, but it is better to employ some automatic device. The 'T' piece may be placed directly in the cork of the bellows. The free limb is connected with a rubber tube which is tied to the handle in such a fashion that it is stepped

on and closed when the bellows are compressed."[1] (Fig. 55.) (The spring may also be placed inside of the bellows.)

R. E. Hall has perfected a simple valve for this purpose (Fig. 56). It consists of a metal "T" piece with a steel plunger, well fitted and oiled, which is driven up by the bellows and falls back in expiration. The excursions are controlled by short pieces of rubber tubing inserted in the brass.

The number of respirations should be about 16 to 24 per minute.

There are many forms of mechanical apparatus on the market for maintaining artificial respiration during operative experiments. One of the most efficient is shown in Fig. 57.

Artificial Respiration Pump (*Dr. Brodie's*).—The pump consists of a piston working in a barrel, 3 in. (76 mm.) in diameter and 11 in. (280

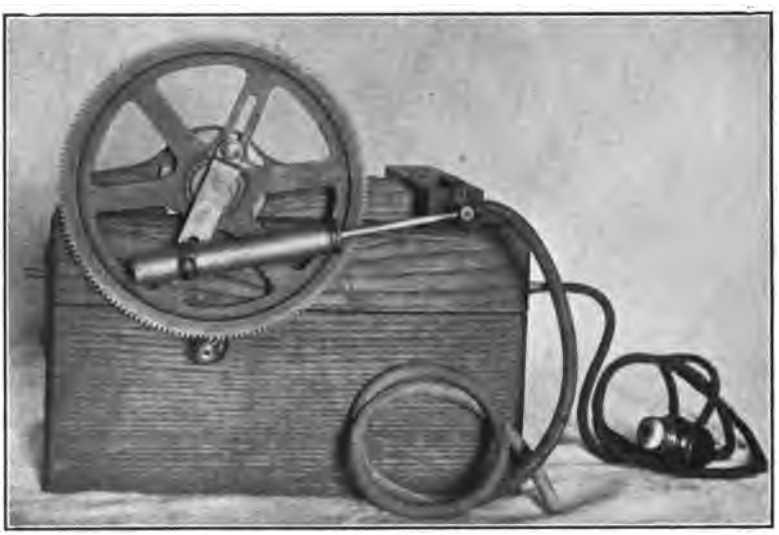

FIG. 58.—Artificial respiration pump for small animals.

mm.) in length. By a simple adjustment of the crank the throw of the piston may be quickly altered to deliver any quantity up to one litre of air per thrust. The pump is driven by a 12-in. three-speed cone wheel, through a friction clutch actuated by a lever for stopping and starting. The valves are placed at the bottom of the cylinder and of the piston respectively. They are of simple construction, and are easily

[1] Sollmann's Text-book of Pharmacology, p. 817.

reached for examination. The upper end of the cylinder is closed in and fitted with an intake tube so that any mixture of gases may be sent to a vertical position on the wall of the experimental room. (See Fig. 57.) the animal. The pump is mounted on a frame, so that it can be fixed in

A cheap and efficient artificial respiration apparatus for small animals such as guinea-pigs can easily be made by mounting a small bicycle hand-pump on a box; the outlet tubing is held stationary while the body of the pump is fastened to one spoke of a wheel near the rim. The wheel is rotated by means of a small motor, enclosed in the box, thus working the pump. The amount of air expelled at each revolution of the wheel may be increased or decreased by regulating the drive of the piston with a set screw. (See Fig. 58.)

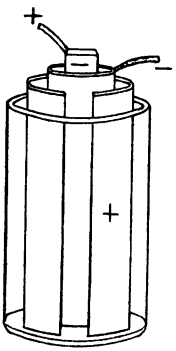

FIG. 59.—Daniell cell.

Batteries.—Either wet or dry cells may be used. The dry cells are the more convenient while the wet cells give a steadier current. The dry cells, however, suffice for most purposes.

(A) *Wet Cells, Daniell Cell.*—"The first constructed constant battery. It consists of a glass jar filled with concentrated solution of sulphate of copper, bathing an unclosed ring of sheet copper around a porous earthen jar filled with sulphuric acid (1 to 10 parts water) in which is immersed a rod of zinc. The zinc pole is the negative or cathode, and the copper pole the positive or the anode, and its electromotive force (E.M.F.) is about 1.07 volts."[1]

(B) *Dry Cells.*—The just described wet cell gives off fumes, contains acids, and must be prepared for use. As the dry cell is always ready and free from the preceding disadvantages it is usually preferred by laboratory workers. The dry cells are usually modified Leclanché batteries. The Leclanché cell consists of a glass jar containing a saturated solution of ammonium chloride into which an amalgamated zinc rod dips. The plate of carbon is fitted into a porous pot packed with pieces of carbon and manganese dioxide. The zinc is negative and the carbon positive. Its electromotive force is 1.5 volts. The dry cell is usually made of a zinc cup lined with plaster of Paris saturated with ammonium chloride. A carbon plate is placed in the center of this and surrounded with black manganese oxide.

[1] Ott's Text-book of Physiology, 3rd edition, p. 575.

Bistoury.—A narrow sharp-pointed surgical knife.

Bull-dog Clamps.—(Artery clips, Langenbeck's forceps.)

Burette.—An accurately graduated glass tube with a stopcock at one end. The burette is used for accurately measuring doses too large to be injected by an all-glass syringe. Also useful in making dilutions.

Colophonium Cement.—Is used for fastening writing points to heart, muscle, respiration levers, etc. This cement is a mixture of colophonium resin and bees wax, which softens at a very low temperature and quickly hardens.

Cannulæ.—(*a*) *Vessel cannulæ* are short tubes of the shapes shown in the following illustrations used for connecting blood-vessels with various apparatus. The small end is tied in the vessel while the larger end is connected with a tube leading either to a recording apparatus or from a burette used for measuring doses.

German silver cannulæ may be purchased from various manufacturers of physiologic apparatus but with a little practice one may easily make his own from glass. First heat the proper size tubing in a blow pipe flame and draw it out in the form of Fig. 60; cool, heat at point *X*

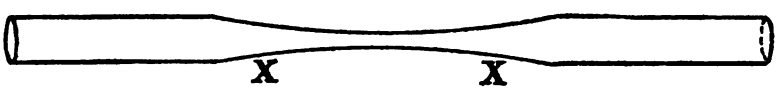

FIG. 60.—Tube drawn for making cannula.

with a *sharp-pointed* flame and again draw gently to the form of Fig. 61; cool, file and break off at 1; put on grindstone and grind off tip to

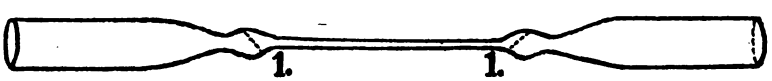

FIG., 61.—Second step in making glass vessel cannula.

dotted line, round off edges in flame.

Cannulæ should be made in various sizes in order to fit the vessels of different animals.

The most important point in glass blowing is to heat evenly the entire circumference of the tubing at the point where it is to be drawn.

One objection to the straight cannulæ shown above is the frequency with which they become clotted. I have found that by using a cannula of the style shown in Fig. 63, this objectionable clotting may be almost entirely eliminated. The superiority of this cannula is due to the fact

that the bulb contains a comparatively large amount of magnesium sulphate or sodium citrate solution, thus giving the blood a greater

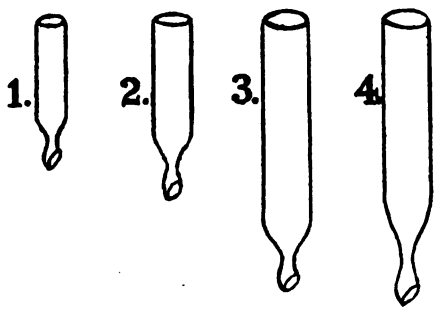

FIG. 62.—Cannulæ for vessels. 1, For guinea-pigs; 2, for rabbits and cats; 3, for medium size dogs; 4, for large dogs.

quantity of liquid with which to diffuse, and thereby increasing the length of time required to produce clotting.

To make a cannula of this type, first, seal one end of a 5-in. piece

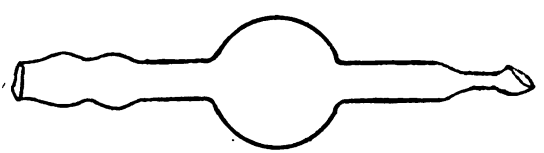

FIG. 63.—Improved vessel cannula.

of glass tubing; heat the central portion to a white heat and blow a bulb about 2/3 in. in diameter; then finish as described on page 110.

(*b*) *Tracheal Cannulæ.*—There are two styles on the market, one of German silver and the other of glass.

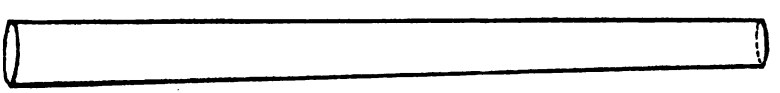

FIG. 64.—Tube drawn for making tracheal cannula.

Tracheal cannulæ can be made from glass as follows: Take a piece of glass tubing about 5 in. long, evenly heat one end in a "fish tail" flame and draw out slightly so that the one end is somewhat smaller than the other. (See Fig. 64.) This will make it possible to use the

cannula for different sized trachea. The two ends should now be ridged to facilitate tying it into the trachea. This is done by evenly heating a small band of the tubing and while hot pushing the ends together. (See Fig. 65.) The tubing is then file-marked, broken off at 1 and 2, and the ends rounded in the flame. The tube is then evenly heated in the middle and bent at right angles.

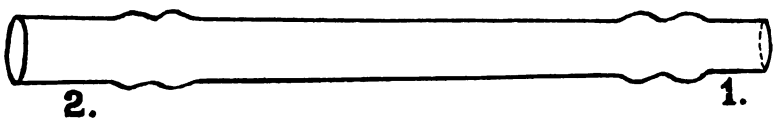

FIG. 65.—Second step in making glass tracheal cannula.

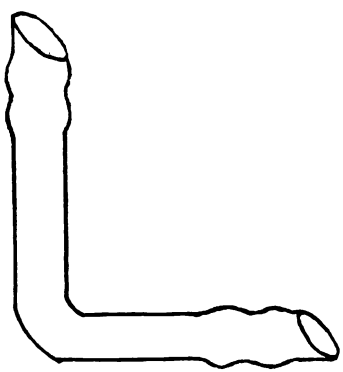

FIG. 66.—Glass tracheal cannula.

Charts. (*a*) *Method of Smoking.*—Charts are best smoked with a luminous gas flame. A burner well adapted to this purpose (see Fig. 67) can easily be made as follows. Take a piece of 1/2-in. gas pipe about 12 in. long, and close one end with a cap; bore holes about 1/16 in. in diameter every 1/4 in. from the capped end to within about 1 1/4 in. of the other end. The uncapped end is then attached to a rubber gas tube. In the absence of gas, charts may be smoked with an oil lamp provided with a wide wick. Heavy oils should be used in order to produce the maximum amount of smoke.

To Smoke Single Drums.—First cover drum with glazed paper and then place upon the sleeve. The sleeve is then twirled between the fingers while holding the drum directly above the flame as shown in Fig. 67.

To Smoke Double Drums.—Remove clock works, then place the frame on the smoking stand and insert the turning crank at the end of the drum rod. Put the long band of paper over the drums and tighten it by moving the rear drum. Revolve the drum by means of a turning crank while holding the gas flame beneath the paper band at such a height that the paper shall pass just below the free edge of the flame. In order to prevent the paper from crawling when the drums are revolved it is necessary to exercise great care in over-lapping the edges of

FIG. 67.—Method of smoking single drum.

the paper where it is gummed. In pasting the ends of the paper together it is best first to lay the paper full length on the table; both ends are then lifted and brought toward each other until they overlap about 1 in. *At this point the operator should see that the edge of the upper layer of the paper coincides throughout its entire length with the edge of the lower paper.* While holding in this position the over-lapping edges of the paper are pasted together.

(*b*) *Method of Fixing Charts.*—Charts may be rendered permanent or "fixed" by passing them through an alcoholic solution of benzoin or varnish. The strength of this solution depends upon the operator's

method of filing his charts. If they are small and do not require folding the solution should be rather concentrated, which will give a fine glossy appearance. If the charts are large and require folding the solution should be rather dilute (just strong enough to prevent the carbon from rubbing off)—concentrated solutions cause the charts to crack when folded, and for this same reason dilute varnish is to be preferred to benzoin.

If the tracings are to be used for making half-tones or zinc etchings for reproduction in journals, etc., the two following precautions should

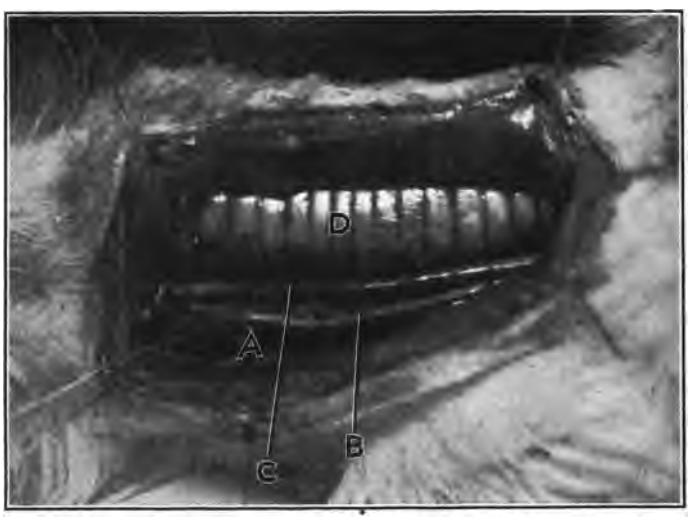

FIG. 68.—Typography of dog's neck: *a*, sternohyoid muscle; *b*, vagus; *c*, carotid artery; *d*, trachea. The carotid artery and vagus have been removed from the sheath and drawn apart.

also be taken: (1) Paper should be smoked as black as possible; (2) fixing solutions should be filtered and be as dilute as possible, as concentrated solutions cause the tracings to turn yellow thereby greatly increasing the difficulty of making the reproductions.

Charts which have become disfigured by use, or scratched, may be retouched before making reproductions by painting over the disfigured places with paint made from lamp-black and turpentine. After this, the charts can be re-fixed. This simple procedure will in most cases greatly improve the appearance of the reproduction.

Carotid Artery, Method of Exposing.—After clipping the hair from the neck a long incision is made in the median line from the thyroid to the breastbone. The edges are then held apart and the position of the sternomastoid and sternohyoid muscles determined. Following the edges of the sterno-mastoid the incision is carried into the groove and the attachments exposed. The carotid artery is at once detected by its strong pulsations. After roughly cleaning, it is lifted to the surface by means of a tenaculum or the fingers. In the dog the vagus, sympathetic and depressor fibers lie in the same sheath as the carotid artery. In the rabbit these run separately but may be recognized by their size—the vagus being the largest, the depressor the smallest. The carotid is then carefully and gently separated from the vagus.

For method of connecting artery with manometer, see page 54.

Chronograph.—See signal magnet, Fig. 88, page 140.

Clotting.—In blood-pressure experiments if the writing point of the manometer ceases to pulsate while the heart beat can still be felt, clotting has occurred, either in the cannula or the connecting tube. In such cases the artery and connecting tube should be clamped and disconnected. Both should then be cleaned and reconnected. Upon releasing the clamps the writing point should again record the pulsations of the heart.

Constant Temperature Bath.—When using frogs for assay purposes it is very important that the experiments should be carried out at the same temperature on account of the great susceptibility of the frog to heat. A temperature of about 20° C. is the best, because, being about ordinary room temperature, it can be easily maintained. This may be best accomplished at all seasons by means of a simple apparatus consisting of a large galvanized iron tank partly filled with water in which are placed the small cages containing the frogs. The temperature of the tank is raised or lowered by merely heating or cooling the water.

A tank of the form shown in Fig. 69 is best adapted to this purpose as it can be utilized in carrying out assays upon frogs according to any of the frog methods of assay so far proposed. The tank is provided with an inlet and outlet pipe for running water. The top of the outlet pipe is about 1/4 in. from the top of tank. A perforated shelf is placed about 3/4 in. beneath the surface of the water. In winter the water in the tank can be brought to the proper temperature (about 20° C.) by placing a small burner under the tank. In summer the temperature of

running water ranges from about 18° to 23° C. so that in most cases it is merely necessary to employ running water.

In assaying a preparation according to Focke's method the lids of the tank are closed and the frog boards placed on top. When using Houghton's or Famulener and Lyon's method the lids are opened and the frog cages placed upon the perforated shelf in the water.

FIG. 69.—Constant temperature bath for frogs.

Defibrinated Blood.—This is prepared by first whipping the blood, and then straining it through cloth.

Electrically Heated Operating Table.—This new table, designed by Dr. Brodie, is larger than the old ones, having a top 51 in. (129.5 cm.)×18 in. (46 cm.)×40 in. (101.5 cm.) high. It is fitted all round with cleats into which the holding down cords can be easily and quickly fixed. Near the center and flush with the top is a copper hot plate, 30 in. (76 cm.)×12 in. (30.5 cm.), heated by means of two electric lamps, each having its own independent switch. Two upright rods, working in slots, are also provided, and will be found useful for many purposes. Dr. Brodie's well-known anesthetic bottle and air warmer (see Fig. 52) is now fitted to these tables with a bent tube projecting

through the top to supply the air to the animal. The table is fitted complete with animal holder, four controlling switches, main switch and plug; also wheels and handles for convenience in moving.

FIG. 70.—Palmer's operating table.

Electric Clock.—An electric clock is used in conjunction with a signal magnet for making *time tracings*. The writing point of the signal magnet is placed in under and exactly on a vertical line with the writing point of the manometer, heart lever or muscle lever as the case may be. The clock at regular intervals makes and breaks the electric current thus causing the signal magnet to mark the time on the smoked chart.

Figure 71 shows the Harvard electric clock. "The pendulum bearing the armature is released by pressing down the bar at the right. With each swing the pendulum turns one of the several toothed wheels past an adjustable contact connected with the signal magnet at the physiologists' kymograph. This contact is made once in 1, 5, 15, 30, or 60 seconds, according to the toothed wheel used. The five wheels

are mounted side by side on a rod, the outer end of which is seen in Fig. 71. When the pendulum begins to "run down" the movable piece hung at the lower end pauses upon a plate borne by a spring between two magnet coils. Contact is thus made and the magnet now

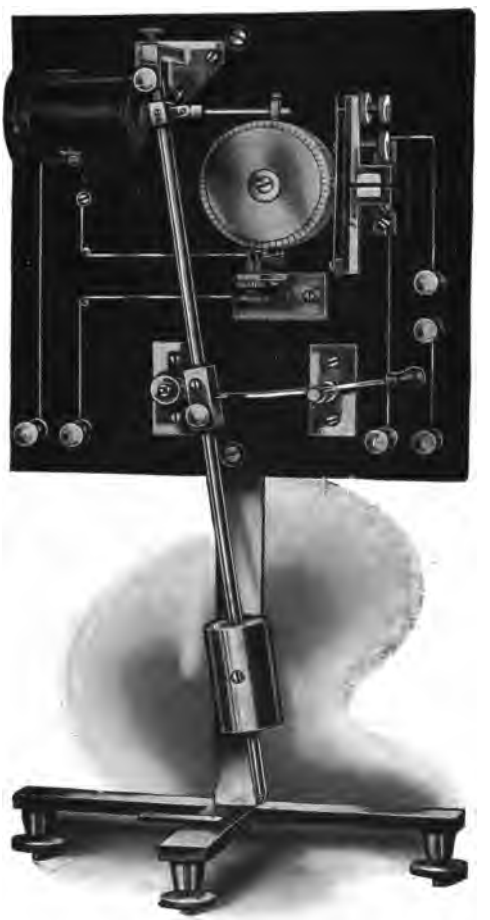

FIG. 71.—Harvard electric clock.

suddenly active pulls the armature, and with it the pendulum up to the full swing, thereby breaking the magnet circuit till the pendulum lags again.

Electrodes.—From his instruction on the theory of electrolytic

dissociation the student should be familiar with the fact that when metal electrodes come in contact with an electrolyte in solution, polarization currents develop. Ordinary metal electrodes in contact with a muscle or nerve will be surrounded by lymph, and in this fluid electrolysis will take place during the passage of an electric current. The ions resulting from this electrolysis will be positive and negative respectively; if, therefore, the circuit of this seat of chemical and electrical change be suddenly made or broken, a shock will be produced, for the wires of electrodes surrounded by the electrolyzed fluid will form a minute battery. This can be demonstrated by the following experiment: A pair of electrodes connected with the DuBois key is placed under the sciatic nerve, which has been exposed in the thigh of the pithed frog. Making or breaking the circuit causes no contraction. The two wires of a Daniell cell are connected with each side of the DuBois key and the current is allowed to pass through the nerve for several seconds. Then these two wires are rapidly disconnected from the battery and key; the key is closed and opened, and each time a contraction of the muscles of the leg is caused. This make and break can be repeated several times with a similar result, until the polarization has disappeared.

This experiment shows the necessity of employing *unpolarizable electrodes* in experiments upon the effects produced in nerve and muscle by the passage of a constant electric current. Electrodes made of metal must for this reason be avoided. Strictly speaking no electrode is nonpolarizable, but practically all the polarization errors are excluded in the "boot electrode." These electrodes are boot-shaped, made of Potter's clay, and were designed by Prof. W. T. Porter.[1] The leg is pierced with a hole 28 mm. deep and 8 mm. in diameter, in which is placed the zinc. The foot is twenty millimeters long measured from its junction with the leg. In the foot is a well for normal saline solution which shall keep the feet equally saturated. In use the hollow leg of the boot is half-filled with saturated solution of zinc sulphate and the rods of amalgamated zinc are immersed in the latter.

The boot electrodes when used are mounted in rubber holders in the apparatus described as the "moist chamber." (See Fig. 86.)

Frogs. *Method of Weighing.*—Frogs can be easily and rapidly weighed by first counter-balancing a frog cage after which the frog to

[1] W. T. Porter: "Science," 1901, XIV, p. 567–570. The well was added in Nov., 1905.

be weighed is placed within the cage, the lid replaced, and the weight determined in the usual manner.

Frog Cages.—A convenient frog cage can be made from ordinary wire test-tube baskets by simply equipping them with tin lids.

Guinea-pig Boxes.—Guinea-pigs are best kept during test periods in small galvanized iron boxes about 9×16×7 in., with lid containing a window covered with wire netting of about 1/4-in. mesh. These boxes accommodate four pigs each. If the boxes are numbered and four pigs all of different colors or markings are placed in each box, the pig which has received any given injection can be distinguished without the objectionable use of tags, labels, etc.

Head Holder.—A head holder is not generally needed in biologic standardization work. It is, however, advisable to use a head holder of some sort when working with cats.

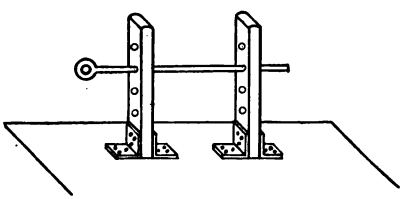

FIG. 72.—Head holder and animal board.

FIG. 73.—The Czermak head holder. About one-half actual size.

A holder of the design shown in Fig. 72 is cheap, easily made, and answers almost every purpose. The neck is placed between the two vertical posts and the bar placed across it. This holder surrounds the neck without compression and the head cannot be withdrawn. When

using dogs the cross piece is pushed back of the teeth; a piece of stout twine is then passed under the neck, behind the ears, the ends are brought forward, wound tightly around the cross piece and tied about the mouth. A more expensive and efficient holder is shown in Fig. 73.

Heart Lever.—This very light lever is used in the suspension method of recording the contractions of the heart, or for similar purposes. The axle is 7 mm. in length. The axle, with its aluminium wire, 22 cm. long, weighs about 0.4 gm.

Hitchens Syringe.—The Hitchens syringe is composed of three parts, *i.e.*, (1) glass body of syringe; (2) needle, and (3) rubber bulb. This syringe is especially designed for administering subcutaneous injections. It is particularly adapted to physiologic standardization work as it allows no possibility of loss while inserting the needle and may be washed with water without being withdrawn. For detailed method of its use see page 27.

FIG. 74.—Hitchens syringe.

Inductorium.—The following illustration shows the Harvard induction apparatus.[1] The primary coil wound with double silk-covered wire of 0.82 mm. diameter, having a resistance of 0.5 ohms, is supported in a head piece bearing three binding posts and an automatic interrupter. The core consists of about ninety pieces of shellaced soft iron wire. This core actuates the automatic interrupter. The interrupter spring ends below in a collar with a screw. By loosening the

[1] W. T. Porter: American Journal of Physiology, 1903, p. 35.

screw, the interrupter with its armature may be moved nearer to or farther from the magnetic core. Once set, the interrupter will begin to vibrate as soon as the primary circuit is made. The outer binding posts are used for the tetanizing current. The left-hand outer post and the middle post are used when single induction currents are desired; they connect directly with the end of the primary wire thus excluding the interrupter. These several connections are all in view; there are no concealed wires.

From the head piece extend two parallel rods 22 cm. in length, between which slides the *secondary coil*, containing 5000 turns of silk-covered wire 0.2 mm. in diameter. Over each layer of wire upon the secondary spool is placed a sheet of insulating paper. Each end of the secondary wire is fastened to a brass bar screwed to the ends of the hard rubber spool.

The brass bars bear a trunnion which revolves in a split brass block, the friction of which is regulated by a screw. The trunnion block is

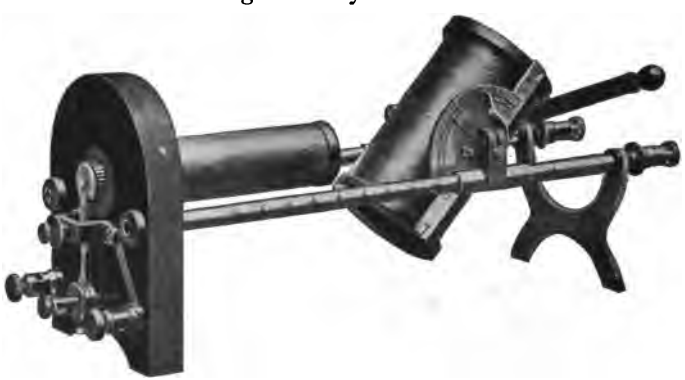

FIG. 75.—Harvard inductorium.

cast in one piece with a tube 3 cm. in length, which slides upon the side rods. A set screw, not shown in Fig. 75, holds the trunnion block tube and the secondary spool at any desired point upon the side rods. This screw also serves to make the electrical contact between the trunnion block tube and the side rod more nearly perfect. The secondary spool revolves between the side rods in a vertical plane. When the secondary coil has revolved through 90°, a pin upon the side bar of the secondary coil strikes against the trunnion block and prevents further movement in that direction. The right-hand side bar bears a half circle graduated upon one side from 0° to 90°. An index-pointer

is fastened upon the trunnion block. One side rod is graduated in centimeters.

The side rods end in the secondary binding posts, so that moving the secondary coil does not drag the electrodes. Next to the binding posts is placed a substantial "knife-switch" short-circuiting key, with hard rubber handle.

Injections. A. **Subcutaneous.**—Are injections made under the skin.

1. *Mammals.*—Subcutaneous injections are best made in mammals by means of a Hitchens syringe. In most cases the abdominal site is to be preferred (for detailed description of method of employing the Hitchens syringe see page 27). By this method absorption occurs more rapidly than when the drugs are given by mouth, the local action on the alimentary canal is avoided, and the operator is more certain to get the full effect of the remedy, provided it is soluble and is not

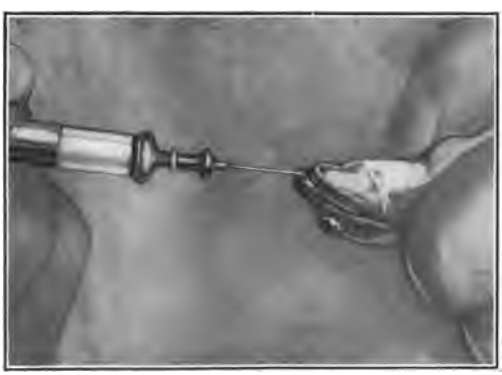

Fig. 76.—Method of injecting frogs.

precipitated at the point of injection. Care should be taken not to pierce blood-vessels, veins or the abdomen with the needle. Irritant drugs should not be injected by this method as they cause great pain, swelling and sometimes suppuration, even when the injection has been carried out aseptically. As the absorption from the subcutaneous tissues is so much more rapid than that of the stomach and intestine, the dose required to produce a given effect by this method is necessarily much smaller than when given by mouth.

2. *Cold-blooded Animals* (frogs).—Due to the lack of elasticity of the skin of this class of animals the perforation in the skin fails to

close upon withdrawal of the needle. It is advisable, therefore, first to pass the needle through muscular tissue in order to prevent the possible loss of part of the injected fluid. In frogs this can be accomplished by passing the needle through the muscle of the leg into the anterior lymph-sac or, better still, force open the mouth, pass the needle through the lower wall into the anterior lymph-sac. By this latter method any fluid which might possibly escape would merely pass into the mouth and be absorbed there. (See Fig. 76.)

B. **Intravenous injections** are injections made into the vein. This is the most certain method of bringing drugs into the circulation and tissues, and is at the same time the most rapid. This method is therefore very largely used in experiments upon animals.

1. *Operated Animals.*—In dogs, cats, rabbits, etc., intravenous injections can be made in either the jugular or saphenous vein. I prefer the latter, however, because being situated at greater distance from the heart it gives the preparation injected opportunity to diffuse more thoroughly with the blood before it reaches the heart.

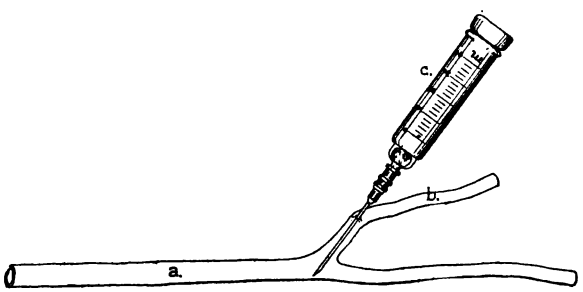

FIG. 77.—Method of injecting. *a*, Femoral vein; *b*, saphenous vein; *c*, all-glass syringe.

Method of Injecting.—The saphenous vein is lifted and held with a pair of tweezers while the needle of the all-glass syringe is inserted far enough through the cannula into the saphenous vein to allow the point to project into the femoral vein (Fig. 77). After injecting the preparation withdraw the needle and quickly clamp saphenous vein with a bulldog clamp.

The advantage of this method is that although the clamping off of the saphenous vein after withdrawing the needle causes clotting, the preparation injected is carried to the heart by means of the main current of blood in the femoral vein.

2. *Intact Animals* (*a*) *Dogs and Cats.*—A ligature is tightly tied above the second joint of one of the hind legs; after the veins have dilated sufficiently to show their location the hair is clipped or shaven from over the internal metatarsal vein; the needle is then inserted, the ligature removed and the drug injected.

A very practical method of making intravenous injections especially for inexperienced persons is given by J. J. Watson[1] which is especially useful in work upon dogs. The limb is corded above the joint

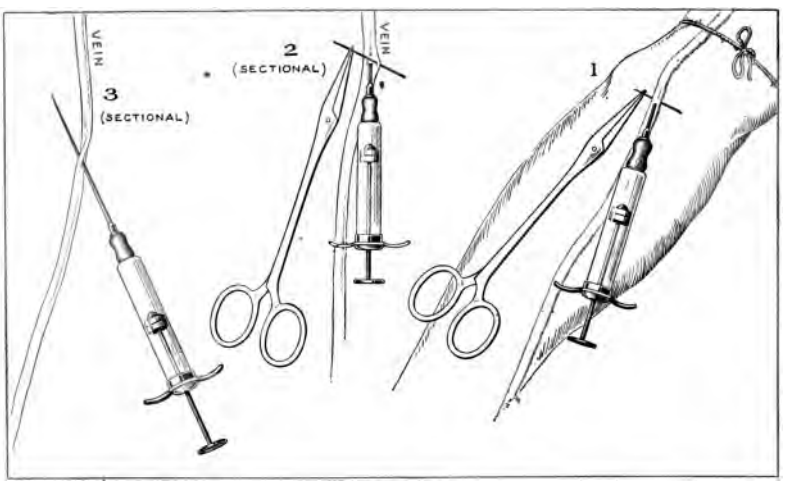

FIG. 78.—No. 1 shows the method of transfixing and raising the vein with a sewing-needle and holding it in the elevated position by means of a hemostat. The syringe needle is shown inserted into the vein beneath the transfixing needle. No. 2 shows more in detail the method of fixation and the insertion of the needle. No. 3 shows what frequently happens in attempting to insert the needle of the syringe without first fixing the vein.

so as to cause the vein to become prominent. The vein is then transfixed with an ordinary sewing needle. The cord may then be loosened and the needle of the syringe inserted into the vein at right angles and beneath the sewing needle which is raised by a hemostatic forceps. The accompanying drawing illustrates the advantages of this method.

(*b*) *Rabbits.*—Intravenous injections are most conveniently made in the ear of these animals. The translucent structure of the ear enables the operator on holding it in a vertical position between himself and the source of light to see the exact location of the veins. It is then comparatively easy to insert a fine needle and inject the drug.

[1] Jour. Amer. Med. Assoc., Vol. LXII, No. 3, p. 383.

(*c*) *Mice.*—In these animals intravenous injections are best made in the tail by means of a fine needle.

C. Intramuscular injections are made directly into the muscles by deep vertical punctures. An ordinary hypodermic needle generally answers the purpose. Care should be taken not to enter any of the viscera, vessels or nerve sheaths.

Insertion of Cannulæ into Vessels.—Cannulæ may be easily inserted into vessels by the aid of a small tenaculum. The pointed

FIG. 79.—Method of inserting cannula into vessel. *a*, Tenaculum; *b*, carotid artery; *c*, cannula; *d*, connecting tube.

end of the tenaculum is first slipped into the vessel through a small V-shaped aperture previously made for the purpose. The aperture is held open by lifting the tenaculum (Fig. 79) and the nozzle of the cannula gradually slipped in. As the cannula is being inserted the tenaculum is gradually withdrawn and the artery then tied fast by means of a ligature.

Isolated Mammalian Heart. *Apparatus Necessary for the Study of Same.*—The mammalian heart isolated completely from the body can be maintained in constant activity for several hours by perfusing

with oxygenated Loche's solution, containing a small quantity of defibrinated blood, at body temperature (38° C.) and under a pressure of about 130 cm. of water, which permits the study of its functions under simple conditions. By adding drug solutions to the perfusion liquid their action may be readily recorded upon the kymograph. The hearts of cats and rabbits are especially well adapted to this experiment; they are preferable to the dog's heart on account of their smaller size.

Preliminary Operations.—As soon as the apparatus has been assembled (see Fig. 80) and ready for use the animal is anesthetized and the carotid artery and femoral vein exposed. The animal is then bled from the artery. As soon as the flow ceases the artery is clamped. The blood is then defibrinated, heated to about 45° C. and poured into the perfusion bottle. The clamp is then removed from the carotid artery and the animal again bled. The maximum amount of blood is obtained by allowing Loche's solution to flow into the femoral vein from a burette. This mixture of blood and Loche's solution is then defibrinated, strained, mixed with the blood first drawn, again heated to 45° C. and replaced in the perfusion bottle. The heart is then quickly excised and tied to the cannula connected with the apparatus; care being taken that the cannula does not interfere with the play of the semilunar valves. The apex of the heart may then be connected with the writing lever by means of a hook-shaped pin and silk thread, or a Guthrie cardiograph may be employed.

When the perfusion is started the pressure closes the semilunar valves, so that the fluid is forced through the coronary circulation, escaping through the right auricle. The solution may be collected in a beaker and the undrugged portions returned to the perfusion bottle and used again. The flow should be rather free. After a few minutes the heart commences to beat feebly and irregularly but soon develops strong regular contractions.

After recording a normal tracing the effects of a drug may be demonstrated by replacing the plain Loche's solution by the medicated solution. After recording the effects of the drug, the heart is again perfused with unmedicated Loche's-blood solution, which causes the heart quickly to return to normal, after which another experiment may be performed.

Apparatus.[1]—"The points to be secured in the isolated heart ap-

[1] Greene's Experimental Pharmacology, 1909, p. 73.

paratus are: 1. A uniform temperature of about 37° Centigrade. 2. An adjustable pressure for the perfusion fluid. 3. A device for quickly shifting from the normal perfusion to the drugged perfusion fluid without change in temperature, pressure, or any other factor than the presence of the drug. 4. An accurate recording device.

"The apparatus shown assembled in Fig. 80 accomplishes all of the

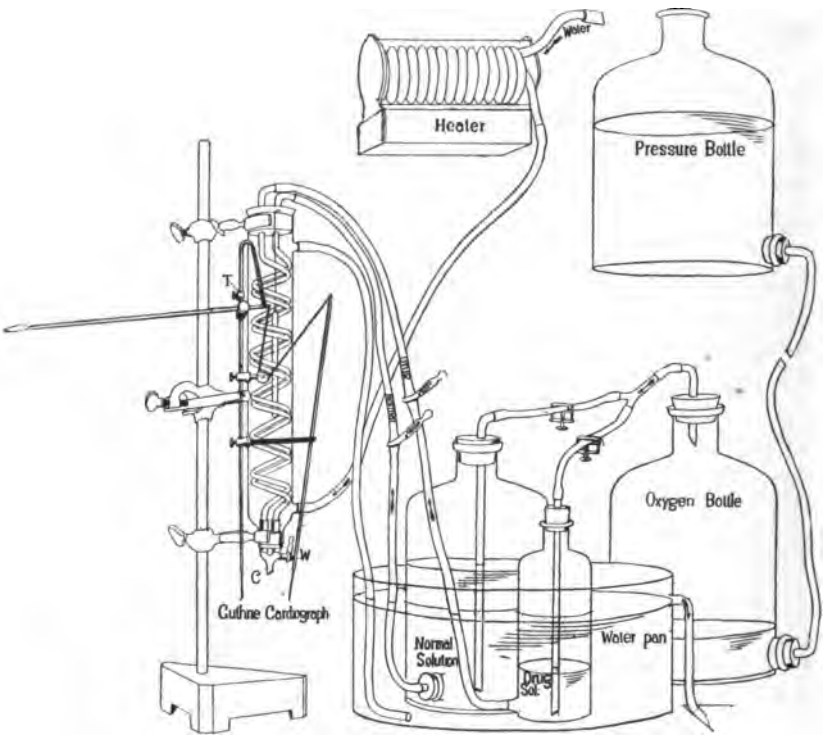

FIG. 80.—Assembly of apparatus for the pharmacologic study of the isolated heart of a mammal. (Greene's Experimental Pharmacology.)

above points. The gas water heater connected as shown will maintain a uniform temperature in the water jacket through which the perfusion tubes run to the heart cannula. The overflow from the water jacket is conducted into a pan in which the perfusion fluid reservoirs receive preliminary warming. The heart is attached to a very short cannula beneath the warming jacket and the overflow of perfusion fluid main-

tains a temperature of the heart only slightly below that of the warming jacket.

"The pressure on the heart, *i.e.*, on the perfusion fluid, is accomplished by connecting the perfusion bottle with an air or oxygen reservoir, and this in turn with a water reservoir which can be raised or lowered. The flow of water from the pressure bottle into the closed system produces the desired pressure on the perfusion system. At the same time the perfusion fluids are aërated by the air (or oxygen) as it is forced into the reservoir, a result accomplished by conducting the perfusion bottle inlet tubes to the bottom of the containers.

"A uniform pressure is secured on both the normal and the drugged perfusion fluids by the system of tubes shown. If the clamp is removed from the outflow tube of the drugged perfusion fluid at the exact moment a second clamp is placed on the tube from the normal fluid reservoir (or *vice versa*), the shift will be accomplished without change of pressure on the heart. The tubes run independently to the cannula which is itself so short that the time from the moment of turning a perfusion fluid on or off is reduced to a minimum. The cannula is provided with a side washout tube.

"The Guthrie cardiograph shown is very adjustable in all essential features. It gives satisfactory and accurate records, if care is used in inserting the lever tips into the walls of the heart. This apparatus permits a direct record on the ordinary kymograph. It also permits one to surround the heart with a warm cup or jacket where greater constancy of temperature is desired, as in research work."

The apparatus described above answers all ordinary requirements, but, for work requiring the greatest degree of accuracy, constancy of temperature, etc., the best apparatus is perhaps the one devised by Eyster and Loevenhart.[1] Their description of the apparatus follows:

"In the study of the action of substances upon the isolated mammalian heart it is of prime importance to remove all variations of temperature and pressure in order to be sure that small changes in rate, amplitude or tone are really due to the action of the drug on the heart and not simple temperature and pressure effects incident to changing from one solution to another. In all forms of perfusion apparatus in which the perfusion fluid is heated by passing through a metallic coil placed in hot water, the temperature which the perfusion

[1] Eyster and Loevenhart: Journ. of Pharmacology and Experimental Therapeutics, Sept., 1913, p. 57.

fluid assumes depends on the rate of flow through the coil. When the outflow becomes slower the perfusion fluid becomes warmer due to

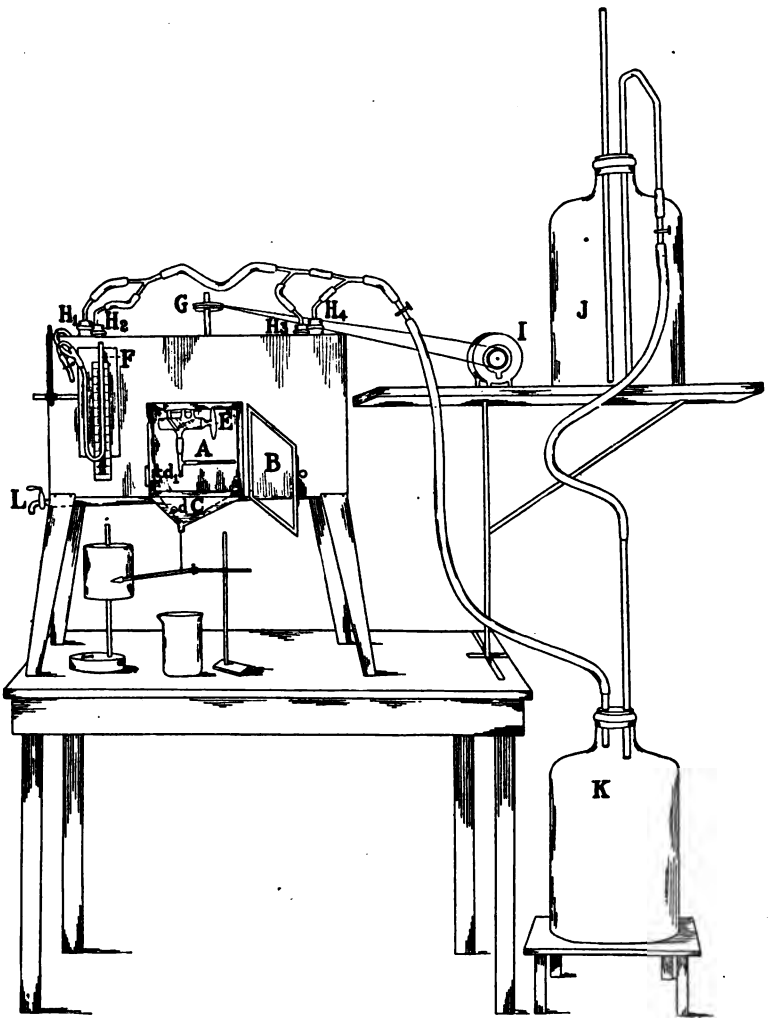

FIG. 81.—Improved apparatus for the pharmacologic study of the isolated mammalian heart. (Side view.) (Eyster and Lovenhart.)

the delay in passing through the coil and when the outflow is increased the temperature of the perfusion fluid falls. Furthermore, the change

from one perfusion fluid to another should be made quickly and all avoidable dead space should be eliminated.

These are the points which we had in mind in designing the apparatus shown in Figs. 81, 82, and 83.

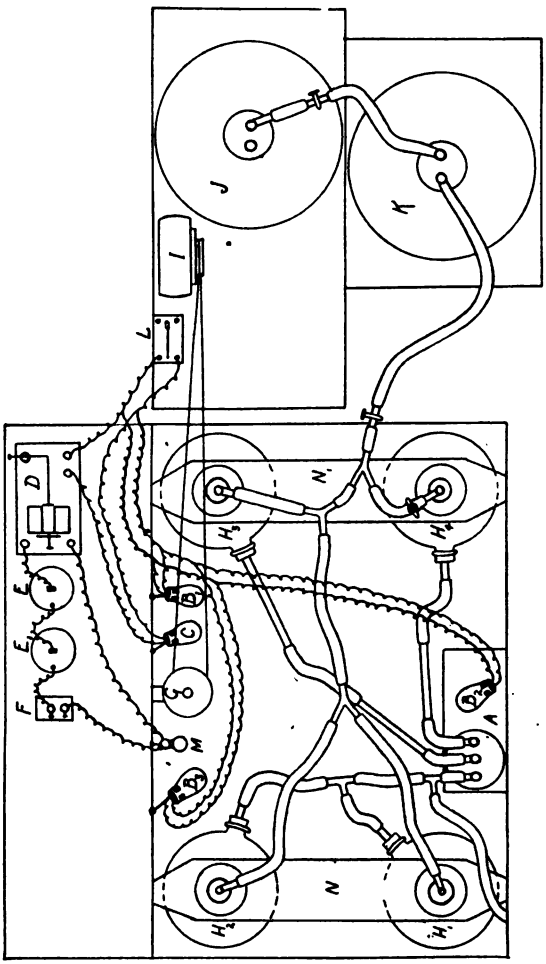

FIG. 82.—Same apparatus as shown in Fig. 81. (Top view.) (Eyster and Lovenhart.)

The tank is of copper, lined with tin. It is 29 1/2 in. long, 18 in. wide and 15 in. deep. The chamber A in which the heart is suspended is 8 3/4 in. wide, 8 in. high and 4 in. deep. This chamber is sur-

rounded on four sides by the water of the tank at the temperature of the perfusion fluid. The glass door *B* in front is on a hinge and the heart can be clearly seen and is readily accessible. The floor of the chamber is funnel shaped so that the perfused fluid may be collected, measured, and analysed if desired, or in case it is desired the fluid may be returned to the bottle and perfused again. Through the opening the thread passes from the heart to the lever. By means of the pulleys *d* and d_1, the auricular beat may be recorded simultaneously with that of the ventricles. The various solutions to be studied are placed in the four bottles H_1, H_2 H_3, and H_4 in the tank and allowed to come to the temperature of the tank. All of the bottles are under the same head of pressure. The bottles are provided with an opening near the bottom (aspirator bottles) which is closed by a rubber stopper. A glass tube passed through the stopper is connected with the four-way stop-cock by means of rubber tubes. Bottles H_1 and H_2 are usually kept for the control solution and are so arranged that either may be connected with the left-hand tube of the four-way stopcock. The manometer *F* is connected with the tube from bottle *H*, and gives the pressure of the perfusion. The glass stopcock is made so that there is no constriction in the stopcock. The bore through the stopcock is 4 mm. and the openings are so placed that we can change from any one of the three solutions to any other one directly. By this means the dead space is reduced to a minimum. As the bottles become empty they are prevented from floating by means of the metal sheets *N* and N_1, shown in Fig. 82. These sheets fit on a runner at either side and are provided with set screws.

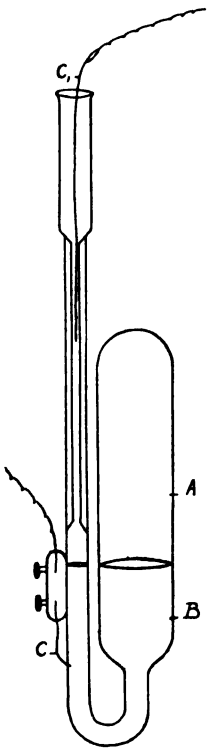

FIG. 83.—Toluene-mercury regulator used to automatically control the temperature of apparatus shown in Figs. 81 and 82. (Eyster and Lovenhart.)

Very active stirring is maintained by means of the fan *G* connected with the motor *I*, by means of a belt. The tank is further provided with a sheet iron bottom which is made in one piece with the supporting legs. This gives greater stability to the apparatus and pro-

tects the bottom in case Bunsen burners are used to raise the temperature of the tank quickly to that desired.

The bottom of the tank is 19 1/2 in. above the table so that a kymograph and other recording apparatus may be placed under it. The faucet *L* is useful in emptying and cleaning the tank. The tank is heated by electric-light bulbs. Three 32-candle-power and one 16-candle-power bulbs may be used according to the temperature of the room and that required for the perfusion. One of these bulbs, preferably the 16-candle-power bulb, is connected with a relay through which the current is made and broken by means of a toluene mercury regulator, Fig. 83. The portion *A* of the regulator is filled with toluene and the portion *B* with mercury. The relay is operated by two dry cells. By this arrangement the temperature of the tank can readily be kept practically constant, the variation being from 0.01° to 0.02° C. Since the entire mass of fluid is immersed in the water of the tank the temperature of the perfused fluid does not alter regardless of the rate of perfusion.

Jugular Vein. *Method of Exposing.*—The external jugular vein may be reached through the same incision made to expose the carotid artery; it is exposed by blunt dissection between the sternomastoid muscle and the skin. It may also be reached directly by a skin incision made about the middle of the neck, in a line drawn from the angle of the jaw to the manubrium.

Kymograph.[1]—"The improved kymograph consists of a drum revolved by clockwork and also arranged to be more rapidly revolved or "spun" by hand.

The drum is aluminum, cast in one piece turned true in the lathe to a circumference of 50 cm. The height is 15.5 cm. and the weight is about 600 gm. The drum slides upon a brass sleeve in bearings 1 cm. deep (to prevent "sidelash") and is held at any desired height by a spring clip. The sleeve ends in a friction plate which rests upon a metal disk driven by the clockwork. Sleeve and friction plate revolve about a steel shaft which passes through the heavy plate containing the clockwork, and is securely bolted to the bottom plate. The sleeve bears upon the steel shaft only by means of "bushings" at the end of the sleeve, thus securing a bearing without "sidelash" and with little friction. As the sleeve of the drum rests upon the friction plate by gravity alone it is easy to turn the drum by hand

[1] W. T. Porter: Introduction to Physiology, 1909, p. 79.

either forward or backward, even while the clockwork is in action. At the top of the sleeve is a screw ending in a point which, when the screw is down, bears upon the end of the steel shaft and lifts the sleeve, and with it the drum, until the sleeve no longer bears upon the friction plate. The drum may then be "spun" by hand about the steel shaft. The impulse given by the hand will cause the drum to revolve for about one minute. The speed during any one revolution is practically uniform.

The clockwork consists of a stout spring about 6 meters in length, driving a chain of gears. The speed is mainly determined by a fan slipped upon an extension of the last pinion shaft in the chain. Four fans of different sizes are provided.

The speed is regulated by a governor on the shaft that carries the fan. When the milled head shown to the right of the steel shaft in Fig. 84 is up, the gear on the extreme right no longer engages with the gear driven by the spring, but runs "idle," while the gear attached to the friction plate engages with the lower of the two gears shown at the left; the pinion of this lower left-hand gear engages with the spring gear. Fast speeds are then obtained.

When the milled head is down, as in Fig. 84, the gear attached to the friction plate falls below the left-hand gear, while the right-hand gear engages with the spring gear and through a pinion drives the friction-plate gear. Slow speeds are then obtained.

These operations are easily and rapidly performed, though, as in all mechanisms, an instant's pause is sometimes required to enable the gear teeth to engage. The clockwork should be in motion without the fan, when the adjustments are being made.

With both fast and slow gearing four fans of different areas may be used. They are slipped upon an extension of the last pinion shaft in the chain. Five slow and five fast speeds (exclusive of spinning) are thus obtained. An additional slow speed (50 cm. per hour) may be obtained with a very large fan.

Long Paper Kymograph.[1]—In Fig. 84 the kymograph is arranged for use with a sheet of smoked paper about 8 ft. long. A rigid bench of steel about 97 cm. long firmly supports two]-shaped frames in which two aluminum drums revolve on pointed adjustable bearings. The rear frame with its drum slides along the bench, and may be fastened at any desired distance from the remaining or clockwork

[1] W. T. Porter, Science, 1906.

drum, so that paper from about 150 to 240 cm. in length may be stretched between the drums. The rear frame is provided with an adjusting screw with which the drum may be inclined until the strip of paper is stretched uniformly throughout its height. When the adjustment is complete the abscissæ drawn by a writing lever in successive revolutions will exactly coincide. The clockwork drum does not slide along the bench. Both drums may readily be removed from their bearings.

Beneath the clockwork drum is a circular plate of the exact size of that of the single drum kymograph. This plate rests on two feet and supports the anterior end of the steel bench (Fig. 84). Upon the plate are three rounded pins. The clockwork drum is driven by a

FIG. 84.—(Harvard) Long paper kymograph. About one-twelfth the actual size.

kymograph, the feet of which are hollowed to fit the three rounded pins just mentioned. When the kymograph is set upon these pins, it is at once "centered" and all side motion is prevented. The vertical steel drum rod and sleeve of the kymograph are replaced by a short rod with a sleeve ending in a collar, the top of which is flush with the upper plate of the kymograph. The collar is pierced with four holes. To connect the clockwork with the drum a coupling sleeve is let down from the shaft of the clockwork drum until four pins on the under surface of the coupler engage the corresponding holes in the kymograph rod.

Langenbeck's Forceps.—See Bulldog clamps.

Ligatures.—The ligatures should not be too thick and should be tied as tightly as possible. Linen thread or button-hole twist answers the purpose for vessels, but lacks sufficient strength unless a very coarse thread is employed. For this reason I find "dental floss" to

be about the best cheap ligature, for vessel work, as it is both fine and of high tensile strength. Twine should be employed for trachea, bladder, etc.

Manometer.—A manometer (see Fig. 21, page 55) consists of a glass U-tube mounted upon a board to which is screwed a rod to be

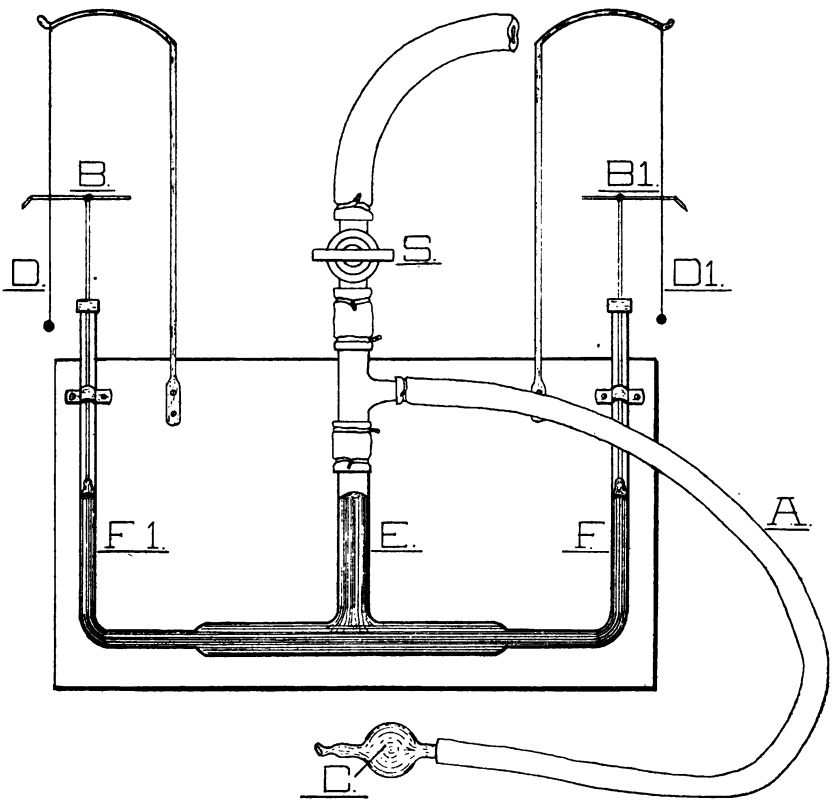

FIG. 85.—Manometer for recording fast and slow tracings at the same time. *A*, connecting tube; *B* and *B*1, writing points; *C*, cannula; *D* and *D*1, guides. Area of tube *E* is twice that of the tubes *F* and *F*1.

clamped in a stand. The one side of the U-tube is connected by a rubber junction to one limb of a glass T-tube. One of the other limbs of the T-tube is connected with a rubber pressure tubing long enough to reach to the carotid cannula. The remaining limb is connected with a rubber junction to a brass stopcock which in turn is

connected by means of pressure tubing with a pressure bottle containing 25 per cent. magnesium sulphate solution. The manometer is about one-half filled with mercury. The connecting tube and one side of the manometer are filled with magnesium sulphate solution by opening the brass stopcock. The end of the connecting tube is closed with a pinch-cock.

The other side of the U-tube bears a hard rubber float, for recording the excursions of the mercury. The float is hollowed to conform with the meniscus of the mercury and should fit the tube snugly but have sufficient play to avoid friction. The float bears a piece of aluminum wire about 20 cm. in length, well centered, which passes through the hard rubber cap at the end of the U-tube. To the upper end of this wire is sealed the writing style which is held against the drum by a guide consisting of a silk thread suspended from a rod and loaded with a small weight.

When measuring tracings it must be remembered that the real rise in blood-pressure is twice that which is recorded, since there are two sides to the U-tube and the needle only passes through a space that represents one-half of the difference of level between the mercury in the two sides.

Manometer for Recording Fast and Slow Tracings at the Same Time.—It is sometimes desirable to take both a fast and a slow tracing of the same experiment upon blood-pressure. This is especially instructive with such drugs as digitalis, aconite, strophanthus, etc. A very satisfactory apparatus for this purpose is a manometer devised by the author and shown in Fig. 85. The illustration is self-explanatory. By reducing the two side arms supporting the float to one-half the area of the middle tube both writing points record excursions of the same amplitude as would be recorded in making a single tracing with the ordinary U-tube manometer. This method is a decided improvement over the common practice of joining two manometers to the same carotid by a T-piece, since by the latter method the writing points do not give a correct reading, but, instead, only record half the increases and decreases in pressure that would be recorded by a single manometer or the apparatus described above. In making a fast and slow tracing at the same time the slow kymograph is run continuously, while the fast kymograph is only run at intervals.

By also employing two signal magnets connected with the same key, the charts can be so marked as to enable the operator to dis-

tinguish any point or part of the two charts which were made at the same time.

Moist Chamber.—The moist chamber (Fig. 86) is an ingenious device to prevent drying of the nerve and consists of a porcelain plate which bears near the margin a shallow groove. In this groove rests a glass cover which for the sake of clearness has been omitted from the figure. To the porcelain plate is screwed a rod, by which the plate may be supported on a stand. Within the glass cover are two right-angle rods. One of the rods carries a small clamp, composed of a split screw on which moves the nut, by means of which the femur of the nerve muscle preparation may be firmly grapsed. The holder for the split screw is arranged to permit of motion in all directions.

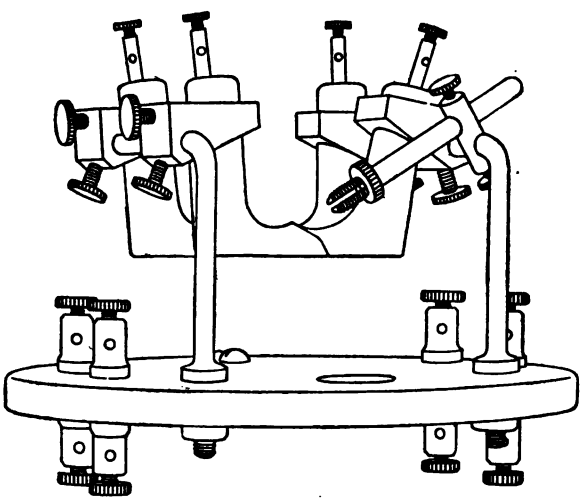

FIG. 86.—The moist chamber; about three-fifths the actual size. (From Porter's Introduction to Physiology.)

Both right-angled rods carry unpolarizable electrodes. Each of these is borne by a hard-rubber holder. By turning the leg of the boot in the holder the foot may be brought as near the foot of the neighboring electrode as may be desired. These boots should be kept in normal salt solution. In use the hollow leg of the boot is half filled with saturated solution of zinc sulphate and placed in the clip. To the hole in the zinc plate is attached a wire which connects with one of the four binding posts shown in Fig. 86. These four posts are in electrical con-

nection with four posts beneath the porcelain plate. The electrodes are made of unglazed potter's clay and have the shape of a boot. The boot electrodes serve equally as well for leading off the nerves or muscles to the electro-meter and for stimulation. After use the boots should be emptied, rinsed with tap water, drained, placed in several hundred cubic centimeters of normal saline solution until wanted again. *If the foot of the boot is kept saturated with normal saline solution, these electrodes will remain non-polarizable.*

The air within the moist chamber may be kept saturated with water vapor by applying moist filter, or blotting paper to the inner side of the glass globe.

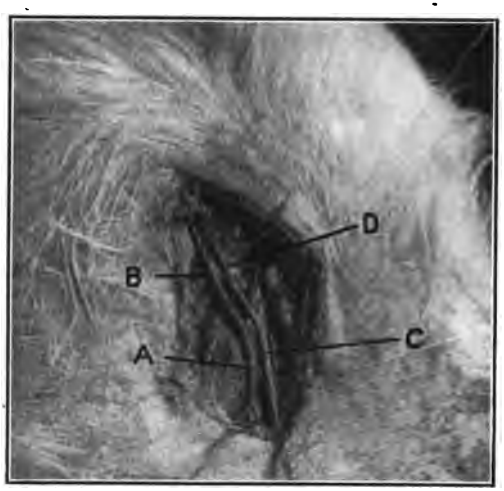

FIG. 87.—Shows method of exposing saphenous and femoral veins: *a*, femoral vein; *b*, saphenous vein, showing its junction with the femoral; *c*, femoral artery; *d*, saphenous artery, showing its junction with the femoral.

Muscle Lever (Light).[1]—A stout yoke bears two set screws holding a steel axle upon which is mounted a light piece of tubing and a metal pulley. One end of the tubing tapers slightly to receive the writing straw. The other projects behind the axle, and may be pressed upon by the accurately cut after-loading screw. The pulley is pierced with a hole for securing a fine wire by means of which a weight may be suspended from the pulley when it is desirable that the weight

[1] W. T. Porter: First catalogue of Harvard physiological apparatus, September, 1901.

should be applied near the axis of rotation. The muscle may also be weighted by means of a scale pan suspended from the double hook to which the lower end of the muscle is attached. If the tendon of the muscle be fastened to the double hook by a fine wire, the free end of the wire may be carried to the insulated binding post provided for convenient electrical stimulation. The upper end of the muscle may be grasped in the flat-jawed clamp and thus connected electrically with the binding post upon it.

Saphenous Vein. *Method of Exposing.*—The femoral vessels may be felt pulsating just below Poupart's ligament on the outer edge of the stiff adductor longus muscle. The artery lies partly behind and external to the vein. The dissection is performed so as to expose about 1 in. of the saphenous vein at its junction with the femoral. (See Fig. 87.)

Signal Magnet (*Chronograph*).—A signal magnet consists of a small metal box (Fig. 88) open at the front and ends, enclosing a strong magnet, the armature of which is mounted upon a steel spring.

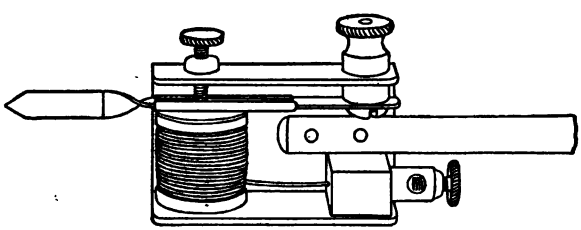

FIG. 88.—The signal magnet; the actual size.

An accurate fine adjustment screw regulates the excursion of the armature. One binding post is mounted upon the metal box, the other is insulated by a rubber block. This signal, in circuit with a vibrating tuning fork, will record 100 double vibrations per second. In the primary circuit of the inductorium it will record the make and break of the circuit without the after-vibration. The handle is long enough to bring the writing point directly above or below the writing point of the muscle lever clamped to the same iron stand.

"Lag" due to residual magnetism is lessened or prevented by parchment paper shellaced to the under surface of the spring over the core of the magnet. The paper should be renewed when necessary.

The signal magnet or chronograph is used in conjunction with an electric clock (see Fig. 71) for making time tracings. The signal

point records on the smoked drum the number of times per second a current through it is made and broken by the clock. The writing point of the signal magnet is placed in under and exactly on a vertical line with the writing point of the manometer, heart lever or muscle lever as the case may be.

The signal magnet is also useful for marking the time of injections, stimulations, etc. The electro-magnet is connected with a battery, with the interception of a key, which is closed whenever a mark is to be made on the drum.

Simple Electric Key.—The simple electric key is used to make and break the electric current. It is especially useful in the study of the effect of stimulation on muscle and nerve muscle preparations. There are several forms on the market, but a small electric-light switch will answer for many purposes.

Tambour.—The Marey Tambour is a very simple but satisfactory instrument to be used in recording the intra-thoracic pressure curve, or for any purpose for which at ambour of this type may be adapted. The moving parts are very light. The membrane may be made of "dental dam" or any similar rubber, not too tightly stretched.

Temperature of Animals.—This is practically always taken in the rectum. In order to secure uniform results the thermometer should always be inserted for the same distance, which may be accomplished by marking the stem.

Tracheotomy.—Is the formation of an artificial opening into the trachea. Tracheotomy is necessary in all experiments on mammals which require artificial respiration. The trachea is freed immediately below the thyroid cartilage and opened. A tracheal cannula (see Fig. 66) is then inserted and tied with a small stout twine.

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CHAPTER VIII

SOLUTIONS

Epinephrine, *Standard Solution:*

C.P. ash-free epinephrine crystals	1.0
Dilute hydrochloric acid	1 drop.
Normal saline solution q. s.	1000.0

Loche's Solution:

NaCl	9.00
KCl	0.42
$CaCl_2$	0.24
Dextrose	1.00
$NaHCO_3$	0.50
Water q. s.	1000.0

All the salts should not be added to the water at the same time as this results in a cloudy solution probably caused by the formation of calcium carbonate, but should be added and dissolved in the order named, in which case calcium carbonate dissolves in the sodium and potassium chloride solution resulting in a perfectly clear solution. The water employed should be glass distilled.

Loche-blood Solution:

Add 5 to 10 per cent. of defibrinated whole blood to the above Loche's solution.

Normal Saline Solution:

For Frogs:

NaCl	7.5
Water q. s.	1000.0

For Mammals:

NaCl	9.5
Water q. s.	1000.0

Perfusion Solutions:

Aconite	0.1	per cent. in Ringer's solution.
Digitalis	1.0	per cent. in Ringer's solution.
Epinephrine	0.0001	per cent. in Ringer's solution.

Ringer's Solution:

For Frogs:

NaCl	7.00
KCl	0.30
$CaCl_2$ (crystals)	0.26
Water q. s.	1000.00

Ringer-Langendorff Solution:

$NaCl$	8.000
$CaCl_2$	0.100
KCl	0.075
$NaHCO_3$	0.100
Water q. s.	1000.000

Ringer-Loche Solution:

Loche's solution with the omission of the dextrose.

PHYSIOLOGICAL LABORATORY

No.....

.....191

Drug.....No.....Received.....

Test employed.....Standard.....

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Amount used to produce desired effect.....

Per cent of activity as compared to standard.....

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Signed.....

FIG. 89.—Sample page from Laboratory Report Book.

Solutions Used to Prevent Clotting.—In blood-pressure experiments the cannula and connecting tube should always be filled with one of the following solutions to prevent clotting.

1. Half saturated (25 per cent.) solution of magnesium sulphate.
2. One per cent. sodium citrate solution.
3. Half saturated (14 per cent.) sodium sulphate solution.
4. Half saturated (12 per cent.) sodium carbonate solution.

Care should be taken not to produce too great a preliminary pressure in the manometer, as ofttimes when opening the screw clamp on the connecting tube this high pressure forces some of the non-clotting solution into the heart. If this occurs prompt paralysis of the heart follows. The effects produced by small amounts entering the heart quickly pass off, larger quantities, however, stop the heart completely. When this occurs artificial respiration, normal salt solution injected into the vein, and cardiac massage, if started at once, will often resuscitate the animal.

In our experience we have found the magnesium sulphate solution to give the best results. The sodium sulphate and sodium citrate solutions are less dangerous but also less efficient. The sodium carbonate solution is about as efficient but is still more dangerous and is therefore not well adapted to this purpose.

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